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A Translation Of

PLANT PHYSIOLOGY

(FIZIOLOGIYA RASTENII)

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PLANT PHYSIOLOGY

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CONTENTS

CONTENTS (continued)

THE 50TH ANNIVERSARY OF THE PUBLICATION OF LENIN'S BOOK, MATERIALISM AND EMPIRIOCRITICISM

P. A. Genkel'

Lenin's book,"Materialism and Empiriocriticism," isto the present day the living source of ideas which materialism draws on in the struggle against idealistic tendencies in science.

This, Lenin's principal work, is of immense significance in the development of the natural sciences, including biology, and especially in the development of plant physiology. In this field incorrect idealistic Kantian views have had a certain influence. Such views have to some extent been utilized by certain scholars in formulating the theoretical bases of problems and methods in plant physiology. They have retained currency even into the 1930's, almost 20 years after the great October Revolution. As an example, one can turn to the definition of plant physiology given by the prominent physiologist and biologist, S. P. Kostychev, in his text, "Plant Physiology" "Plant physiology is the study of external manifestations of the vital properties of the organism." (2nd ed.,chap. 1, p 14, 1933). In this definition the influence of Kant's philosophy — a critical idealistic philosophy of the known world of phenomena and the unknown world of things-in-themselves — is clearly manifest.

It should be pointed out that the phrase " a thing-in-itself" is here used in the Kantian sense, and not in Engels' materialistic sense. In "Ludwig Fuerbach," Engels expresses himself on this point as follows, "The most decisive rebuttal of these, as of all other philosophical fallacies (or lies, Schrullen) resides in practice, specifically,inexperiment and in industry. If we can demonstrate the validity of our interpretation of a given phenomenon of nature by reproducing it, by isolating it, by compelling it, moreover, to serve our purposes, then the Kantian notion of 'things-in-themselves', which are to be neither comprehended nor harnessed, becomes absurd. Chemical substances produced by plants and animals remained such 'things-in-themselves' until organic chemistry began to synthesize them one after the other; a 'thing-in-itself; then became a 'thing-for-us', as, for example, alizarin, the dyestuff from madder, which we now obtain not from the natural source, madder roots, but much more cheaply and simply from coal tar." (p 16, loc. cit.), (Lenin, 4th ed.,v 14, p 89). Fortunately for the development of plant physiology, Kostychev took a basically materialistic point of view in his own researches and laid bare the true essence of the phenomena he studied, i. e., he assumed a position of naive realism, as Lenin said in speaking of Mach, "The example of Mach's reasoning which we have just cited, as well as a number of other examples of fragmentary proofs, are illustrative of so-called naive realism," i. e., the materialistic theory of knowledge unconsciously absorbed from natural scientists. (Lenin, 4th ed., v 14, p 53).

Turning to Kostychev's investigations, it should be pointed out that his discovery of the existence of the Cannizarro reaction in yeast led him to clarify the significance of the role of acetaldehyde as one of the final links in the chain of events comprising alcoholic fermentation. Further development of his hypotheses by a number of workers made it possible to actually divert fermentation along desired pathways. By trapping acetaldehyde with sulfites, the synthesis of considerable amounts of glycerin was achieved. This was of enormous practical value to Germany in the synthesis of nitroglycerin during the first World War, since she was unable to obtain fats because of the blockade.

Of equal importance are Kostychev's studies on the genetic relation of respiration to fermentation, and other outstanding studies.

That Kostychev was not fully committed to his stated philosophical opinions but in practice followed much more progressive views is evident at least in the following statement, "It is impossible to ignore the energy and

unshakeable faith in the ultimate success of those scientists who do not succumb to corrosive scepticism which, with an ironic smile, hastens to proclaim the futility of our efforts to unravel the perplexing enigmas of nature and reiterates again and again the impermanence of our scientific gains. In spite of this unhealthy tendency, which has discouraged more than a few of our young talents from science, the course of scientific progress shows that the most difficult problems were solved after the application of the most potential of science — the experimental method." (p 12*).

It should be emphasized, however, that the main line of development of Russian, and subsequently of Soviet science, was along the line of a materialistic interpretation of nature.

Even in the 1880's K. A. Timiryazev was placing the problems of plant physiology on a materialistic footing. He wrote, "The purpose of plant physiology is to study and explain the vital phenomena in the plant organism, and not only to study and explain them, but by these means to subject them to the wise control of man so that he may at will alter them, suppress them, or elicit them." This definition became the basis of study and development of plant physiology in our country.

How timely and how appropriate are these words in our day, when our scientists, having mastered the principles of dialectical materialism, are penetrating into the essential nature of phenomena which may then be harnessed to serve the uses of our people in their struggles to build communism.

Mach's idealistic study of the reality of elements (sense-perceptions), in effect, the negation of the existence of a real world outside subjective perceptions, led him to deny the existence of the atom, to repudiate the physical picture of the world. Having shown that the discarding of old interpretations did not shake the foundations of materialism, but only strengthened them, Lenin proved, philosophically, the inexhaustibility of the atom and showed the objective meaning of the change in theory which took place in physics at the beginning of the 20th century as a result of several major discoveries. In effect, the philosophical solution of a number of problems stemming from the most recent successes in physics, problems relating to thermonuclear reactions and to world utilization of atomic energy, only confirm the validity of Lenin's profound conception of the inexhaustibility of the atom and of the objective nature of phenomena occurring in the world around us.

It is interesting that, as early as 1911, Timiryazev perceived the reactionary meaning of Machism, as expressed in these words: "If they blamed Kant (as we saw, without any basis) because for 30 years he did not foresee the discovery of the spectroscope, then, what is to be said of Mach who, seven years after the discovery of the scintillascope and a year after the conclusive triumph of atomism, in answering Planck, who had expressed the perfectly obvious view that the physicist had as much right to speak of the weight of the atom as the astronomer to speak of the weight of the moon, permits himself a sally of such dubious wisdom as, 'If faith in atoms is so essential to us, then I renounce the physicist's ways of thought; I do not wish to be an honest physicist, I abstain from any assessment of scientific values, I do not wish to remain in the community of believers; freedom of thought is more important to me.'"

"What biting words!", says Timiryazev further. "Freedom, from what? from a carefully documented fact refuting a pet philosophical theory? And yet Mach has recently asked his readers to regard him as a scientist, not as a philosopher. Of what consequence is this scoffing at physicists, this beration of their community of believers on the lips of a man who had at one time retired from the ranks of physicists to become a follower of His Grace, the Bishop of Cloyne (Berkeley)." (K. A. Timiryazev, Works, v IX, pp 130-131).

As these quotations show, the greatest plant physiologist of our country understood clearly, even then, what an adverse effect Machism has on the development of science.

Unfortunately Lenin's brilliant book became widely known to the majority of natural scientists only after the October Revolution. Nevertheless, such was its significance that it has continued to play down to the present day a prominent role in the interpretation of natural phenomena. The only thing to be regretted is that biologists do not utilize Lenin's thought to a sufficient extent in the study of actual physiological processes in the organism.

The inexhaustibility of biological phenomena and the qualitative uniqueness of vital processes should be considered of paramount importance in the study of living organisms. A mechanical description of physiological

*Plant Physiology, 1st ed., OGIZ, 1924.

laws in terms of physical and chemical laws cannot be considered valid; such a procedure is manifestly in the subjective-idealistic tradition. On the other hand, a thoroughgoing study of vital processes by methods of contemporary physics and chemistry does not cramp our conceptions as to the inexhaustibility of vital phenomena but on the contrary extends them. The invention of the electron microscope made possible the actual demonstration of viruses and bacteria. The use of ultrathin sections for the electron microscope placed in biologists' hands a more powerful means of studying the ultrafine structure of living organisms and of relating structure to function in such organelles as muscle fibers and chlorophyll grana. The use of labeled atoms in biological research made it possible to study metabolic processes, for example water movement, translocation, chlorophyll turnover, etc., as they were taking place in the organism.

Methods such as paper partition chromatography made it possible to detect metabolites occurring in concentrations too low to be detected by ordinary methods of chemical analysis.

The isolation of cell organelles and detailed study of their function has also become possible in recent years. All this should lead us to a more profound knowledge of the plant's life.

The constant deepening of our understanding of the vital processes of a plant will eventually lead to a greater mastery of these processes and finally to a practical utilization of the knowledge obtained.

Dialectical meterialism teaches that practice is the criterion of validity. The more profound and significant a theory, the more fruitful it is in practice.

Unfortunately,many biologists are under the impression even in these times that a practical investigation is also theoretical in scope. They believe that if a scientific study touches on even a small fact, but one which does not give rise to practical results, then this study may be considered theoretical. In point of fact, such a study may only be described as one which describes or establishes a new fact. Such studies make up the bulk of scientific work. Undoubtedly they are important since they increase the total sum of knowledge, but ordinarily they have no theoretical significance and do not play a decisive role in the development of scientific hypotheses.

A truly theoretical study, one which answers questions of basic significance, is always closely connected with practice. Very often, however, this is completely misunderstood and the theoretical implication of studies closely related to practice is ignored. This has frequently led to an improper application of theoretical studies. For quite a long time ,many people regarded the theory of orderly development by stages merely as a blueprint for vernalization techniques, not realizing that vernalization is but one application of this theory of development of biological organisms.

Hypotheses as to the advantage of withholding the first water application in irrigation agriculture were artificially constructed on the basis of interesting facts concerning the increase in photosynthetic rate associated with a slight wilting. It is to be expected that a "theory" of this sort could play only an injurious role in practical work.

A problem which has always puzzled scientists should also be considered. In many cases a theoretical subject of large scope does not immediately find applications. In such cases, where there are no practical results forthcoming at a given time, but where basic questions of theory are being answered, the subject should be carefully considered and given the fullest scope for development. In the history of science there are numerous instances of this. There is, for example, the subject of spontaneous generation, which, as we know, assumed a colossal practical significance in medicine, the food industry, etc., after the work of Louis Pasteur. In our day the problem of the origin of life could occupy a similar position. One has only to refer to the international symposium, "The Origin of Life", or to A. I. Oparin's book of the same title to be convinced that such is not the case. Here,the intimate relation of these problems to important problems in virusology, heredity, etc., becomes immediately obvious.

Thus, the actual study of the nature of evolutionary processes is constantly producing far-reaching practical consequences.

The immediate problem in plant physiology as defined by the XXXIst congress of CPSU is to seek out all possible practical applications of theory. The materialistic philosophy has always been consistent with the study of natural phenomena from a dynamic point of view (development) rather than a static point of view. In this connection, the role of K. A. Timiryazev, who constantly emphasized the necessity of using the historical method

together with the experimental method in plant physiology, cannot be forgotten. He himself, as is well known, furnished brilliant examples of the use of the historical method in the explanation of certain properties of plants (why they are green, etc.). This approach was further developed by other scientists such as B. A. Keller, V. N. Lyubimenko, and most recently by I. V. Michurin, T. D. Lysenko, and others with excellent results. Here, in the Soviet period, the direct effect of Lenin's remarkable book on the efforts of the writers to combat the infiltration of idealism into biology, and especially into plant physiology, was manifested.

The idealistic philosophy has had a much greater influence on the direction of scientific thought in the West. Studies in which it has been asserted that the historical approach is not necessary in biology have been appearing till very recently. Ungerer's book, "Plant Regulation", the 2nd edition of which appeared in 1926*, is particularly reprehensible in this respect. In accordance with subjective-idealistic trends, he denies that the historical method is indispensable to biology.

Let us recall how insurmountable is the obstacle, as Lenin showed, which the question, whether nature existed prior to man, places in the path of Machism. This is an extremely difficult problem for subjective idealists since in their view the surrounding world represents not a real fact but only a complex of sense preceptions.

Ungerer resolves this problem much more simply; he denies that it exists, as the following statement shows, "In the explanation of processes the transfer of problems to the domain of evolution is as invalid here as in any other branch of natural science." In other words, he completely denies the concept of an evolutionary development of the organic world.

Ungerer is quite right in asserting the necessity of understanding the activities of the plant taken as a whole, but absolutely wrong in speaking of the unavoidable necessity of invoking teleological principles. It is to be expected that for one who repudiates organic evolution only the teleological explanation remains.

Under the term, harmony, he subsumes the plant's normal processes, and under the term, regulation, its responses to abnormal conditions. He fails to understand the adaptive character of such responses.

Ungerer attempts to improve on the vitalism and mechanism of his teachers, Driesch and Klebs, and to base his point of view on the conception of expediency in the organic world, but clearly he is not successful. The following words of Lenin are very much to the point in this connection, "In complete accord with the thought of Marx and in close collaboration with him, Engels, in all his philosophical works, discusses every question in terms of the antithetical interpretations stemming from the materialistic and the idealistic views; neither in 1878, nor in 1888, nor in 1892, does he seriously consider the endless attempts to improve on this system, to advance a new philosophy, whether it be 'positivism', 'realism' or some other professorial charlatanism." (Lenin, 4th ed., v 14, p 323).

Notwithstanding the fact that Ungerer considers his system not to be metaphysical for the strange reason that it is free of hypotheses, his system is idealistic and abstract. His approach to the organism as a logical system, his denial of the evolutionary aspect of phenomena, and his absolutization of expediency observed in nature render his point of view completely inadmissible. His sophisticated teleology remains in essence the same old teleology with which Bacon struggled.

The undervaluation of the evolutionary doctrine led still another German scientist, H. Walter**, to commit the same errors in method. In his book, in the course of a discussion of the plant's reaction to drought, he defends the view that the teleological and causal interpretations of plant adaptations are of equal validity.

He evidently accepts a teleological explanation after having been convinced that static causality, the only kind he knows, cannot give him the answer to the problem of the origin of adaptations. It is to be expected that this mechanistic causality would not satisfy him since it does not reveal the evolutionary pattern of origin and development. However, in accepting teleology, he does not come closer to his goal but is further than ever from it.

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*Ungerer, E. Die Regulationen der Pflanzen. 2-the erweit. Auflage. Berlin J. Springer, 1926.

**Walter, H. Die Anpassungen der Pflanzen an Wassermangel Naturw. und Landw. H. ...Freising – München, 1926.

In the second volume of his textbook on plant physiology, written in German*, S. P. Kostychev asserts that biological science must introduce the idea of teleology since it is only in this way that it is possible to to speak of organized structure and to predict changes in these structures. In his opinion, it is impossible to dispense with the idea of expediency, the analysis of which was given by Kant. Objective expediency, according to Kostychev, is a creation of the intellect and is the basis of our logical hypotheses regarding natural phenomena. It is concerned with organized things only, i.e., with systematic creations of man and of living beings.

Kant describes two types of objective expediency: relative and internal. Relative expediency refers to the relations among organisms and internal expediency to relations within an organism. Here Kostychev, following in Kant's footsteps, approaches vitalism and notes that, in his opinion, "the successes of vitalism" (quotation marks ours — P. G.) are related to the fact that its opponents deny the existence of internal expediency. Kostychev, it is true, says that vitalism does not explain uncontrolled forces, which he admits exist, but he suscribes to these views, as might be expected, and accepts the idea of the occurrence of internal expediency in organisms.

In conclusion, he says that the concept of teleology is entirely admissible in biological science, and that we must recognize that there is an expedient organization in living things. It goes without saying, he continues, that a simple organization (obviously an expedient one) should also occur in protoplasm.

We have discussed only a few examples of how a misunderstanding on the part of many biologists of questions raised by Lenin in "Materialism and Empiriocriticism" leads them into a creative cul-de-sac and hinders the development of science. The wealth of ideas contained in true dialectical materialism, which was developed and defended by Lenin, will for many years to come illuminate the correct path of development of science. The best guide for every natural scientist will always be the enduring words of Lenin "If the world is a self-moving material, it can and must be studied unceasingly in the endlessly complex and detailed manifestations and ramifications of this movement, the movement of this material, but outside it, outside the 'physical', external world known to everyone, nothing can exist." (Lenin, 4th ed.,v 14, p 329).

*As is well known, Kostychev wrote the second part of his textbook in German for publication by Springer, and it was subsequently translated into Russian.

THE RESPONSE OF SESAME TO LIGHT INTENSITY

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As yet the effect of daylight of various intensities on plants in different stages of development has not been adequately described [1]. In particular, the effect of light intensity on fruit and seed formation and on fat synthesis has been studied very little [2]. The main properties of plants involved in their adaptation to light are: light preference and heat resistance, and shade preference and heat sensitivity [3, 4]. The significance of light intensity in the life of plants has been considered in many studies [5-10]. A relation between sensitivity to light intensity and plant ontogenesis has also been shown. Those periods of development characterized by a high light requirement have been established [6, 7, 10, 11, 12]. etc.

The beneficial effect of temporary shading during the period from formation of flower primordia to appearance of pollen tetrads has been previously shown with flax and sesame. It turned out that under these conditions, in addition to changes in productivity, there were appreciable changes in the oil content of the seeds.

In this study we have attempted to determine the causes underlying variations in economically important properties, using light of various intensities (from 52.4 to 6.8 thousand lux). We have investigated the response of Sesamum indicum L. (var. VNIIMK 78) to light intensity.

METHODS

Seeds were sown in rows with a distance between rows of 70 cm. At the time of thinning, two plants were left every 10 cm. At the differentiation of the terminal meristem, which occurred on the 11th day after sprouting, when the plants were 1.5 cm high and had four leaves, they were divided into four groups: the first received no treatment; the second was shaded permanently with 1, 2, and 4 layers of gauze giving 24.8, 14.7 and 6.8 thousand lux illumination respectively, the normal intensity of daylight being 52.4 thousand lux. Light intensity was measured with a light meter at the upper position of the selenium element. Plants of the 3rd, 4th, and 5th groups were temporarily shaded in a similar manner at the following stages: plants of the third group — from the laying down of the flower primordia to the formation of the pollen tetrads; plants of the fourth group — from the formation of pollen tetrads to the appearance of the first flower; plants of the fifth group — from the appearance of the first flower to the end of the experiment.

Climatic conditions were evaluated on a control plot, and also under one and four layers of gauze. Attention was given to the sensitivity of sesame to light intensity during the time of formation of floral organs (microscopic control) since many plants manifest a high sensitivity to external conditions during gametogenesis [6, 10, 11].

Characteristics of Growth and Development of Sesame (Variety VNIIMK 78) under Varying Light Intensities

With permanent weak shading at 24.8 to 14.7 thousand lux, tall plants with elongated internodes, dark green leaves and rather pale flowers were formed. Plants grown in permanent strong shade, at 6.8 thousand lux, were shorter and had sparse dark foliage, elongated internodes and smaller flowers and leaves. The rhythm of development of shaded plants was disrupted, the rates of formation of floral organs were reduced, and the period of flowering and seed ripening was prolonged.

Sesame plants are very sensitive to light intensity during the period from differentiation of the terminal meristem to formation of pollen tetrads, at which time there are from 4 to 16 leaves. Sensitivity to bright light declines during formation of floral organs. The lack of substantial differences in the height of plants before flowering testifies to this (Table 1).

TABLE 1

The Effect of Permanent Shading on Growth and Development of Sesame Var. VNIIMK 78 (M-20)

Items assessed	Light intensity, in thousands of lux			
	no shading, 52.4	24.8	14.7	6.8
No. of days from appearance of flower primordia to formation of pollen tetrads	14	17	17	22
Stem height, in cm, at the time of formation of the first pollen tetrads	15	21	28	44
No. of leaves at the time of formation of pollen tetrads	10	10	10	10
No. of days from formation of pollen tetrads to commencement of flowering	12	11	12	21
Stem ht., in cm, at commencement of flowering	81.3	84	86	88.5
No. of leaves at commencement of flowering	32.5	28.1	28	30

With reduction of light intensity to 6.8 thousand lux, the formation of floral organs is very much slower. Flower abnormalities of the reversion type are also encountered. It may be supposed that sesame plants receiving insufficient light are compensated for this by its action over a long period of time. Thus, the behavior of shaded long day (flax) and short day (sesame) plants is exactly the same: they compensate for a light deficiency by prolonging the stages of development [10, 11]. It has also been shown that the active life of leaves is prolonged by shading.

The temprorary shading from the appearance of flower primordia to the formation of pollen tetrads lasted from 14 to 22 days (third group of plants); a reduction of light intensity exerted a profound effect on the subsequent growth of the plants – the time of flowering was shifted appreciably (Table 2) and the growth of the stem was more vigorous, the difference in plant height being maintained even after completion of the shading period.

TABLE 2

The Effect of Temporary Shading (from Formation of Flower Primordia to Formation of Pollen Tetrads) on Growth and on Time of Flowering in Sesame

Items assessed	Light intensity, in thousands of lux			
	no shading, 52.4	24.8	14.7	6.8
Date of Formation of pollen tetrads	25/VI	28/VI	28/VI	3/VII
Stem height, in cm, at the time of formation of the first pollen tetrads	15	21	28	44
No. of leaves at the time of formation of pollen tetrads	10	10	10	10
Date of commencement of flowering	7.VII	11.VII	12.VII	30.VII
No. of days from formation of pollwn tetrads to commencement of flowering	81.4	81.3	80.2	102
Stem ht., in cm, at commencement of flowering	32.3	32.5	30.1	50.5
No. of branches at commencement of flowering	1,0	1,5	0.4	0.4

Plants of the third group were left unshaded after the formation of pollen tetrads, and for the duration of the experiment were grown under normal light intensities. The time of flowering of these plants was, however, considerably altered. The greater the amount of shade, the more flowering was inhibited. Thus, flowering was delayed to an extent directly proportional to the degree of shading. With temporary shading during the period of formation of floral organs (fourth group), flowering times were the same even when the plants received only 6.8 thousand lux (Table 3). It is obvious that, after gametogenesis, sesame is less sensitive to light intensity.

TABLE 3

The Effect of Temporary Shading during Formation of the Floral Organs on Growth and on Time of Flowering in Sesame (M-20)

Items assessed	Light intensity, in thousands of lux			
	no shading, 52.4	24.8	14.7	6.8
Date of commencement of flowering	7/VII	8/VII	8/VII	10/VII
Height of stem, in cm	81.4	81	84.6	81
No. of leaves	32.3	24	28.5	27.8
No. of branches	1	0.4	0.4	0.7

Concerning the effect of shading on the foliage, the data of Tables 1-3 are of interest. With permanent shading at 24.8 to 6.8 thousand lux, the number of leaves decreases in proportion to the extent of reduction of light intensity. The most heavy foliage occurs with a temporary weak shading (24.8 thousand lux) at beginning stages. As is well known, this period, which immediately follows the light stage, is characterized by an intensification of all the biochemical processes in the plant. It must be assumed that weak shading promotes to a greater or lesser degree the prolongation of the period of renewed growth and the increase in stem height and leafiness.

Plasticity of the Sesame Leaf during Shading

The capacity of a structure to vary in order to adapt to the environment has been repeatedly investigated. In this connection, the anatomical studies of a number of workers merit special attention [15-21]. The main object of these studies was the leaf. However, the history of anatomical studies teaches one to be extremely cautious and critical in selecting a particular feature of a structure as a basis for determining the biological foundation of physiological processes.

The leaves of unshaded plants and those receiving a temporary weak shading during the time from differentiation of the terminal meristem to formation of pollen tetrads possess the largest number of stomates (Fig. 1). In weak light (6.8 thousand lux), the number of stomates in the lower epidermis is halved.

A study of the size of the stomates under weak illumination (6.8 thousand lux), using xylol and alcohol, showed a decrease in the number of open stomates. For example, on August 29 there was a decrease of 30% in open stomates under normal light intensity, while with shading there was a decrease of from 24.8 to 48.2%. Changes in stomate size may be correlated with changes in evaporation.

Plants which have grown in normal daylight have thicker leaf blades with thick veins, a well-developed palisade layer and a thick cuticle (Fig. 2). A strongly shaded leaf (6.8 thousand lux) is characterized by small cells. The tendency of cells of the spongy layer to be in close contact with one another is strongly modified as a result of which the volume of intercellular space is greatly increased. The small-celled structure of the sesame leaf developed under weak illumination cannot be regarded as a sign of xeromorphism inasmuch as there are clearly expressed indications of a mesomorphic structure: cell walls are quite thin, obliteration of intercellular spaces is incomplete, the volume ratio of intercellular spaces of the spongy tissue to the cells is very large, and the spongy tissue is sharply delimited from the palisade tissue. Particularly striking is the almost complete absence in the "shaded" leaf of glandular hairs, so characteristic of all parts of the sesame plant.

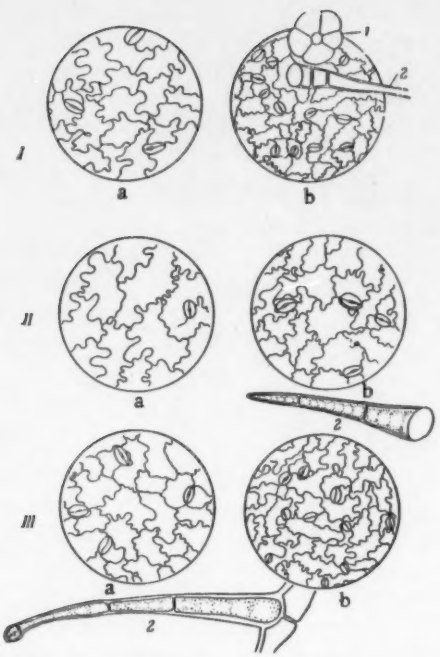

Fig. 1. Upper (a) and lower (b) epidermis of sesame leaves at various light intensities (magnified 280×). Intensity of light given during the period from differentiation of the terminal meristem to formation of pollen tetrads: I) 52.4 thousand lux, II) 6.8 thousand lux, III) 24.8 thousand lux; 1) glandular and 2) simple leaf hairs.

We also followed the rate of outflow of assimilates from the leaves of shaded and unshaded plants. Those leaves were used in whose axils were borne the lowermost capsules. They were collected at 5 A. M. and 12 noon and decolorized with a concentrated solution of chloral hydrate in which iodine was dissolved. Great differences in starch content were found in relation to the treatment of the plants. There was a marked reduction in starch content of weakly shaded leaves (and 24.8 and 14.7 thousand lux).

In strongly shaded leaves (6.8 thousand lux), the starch content was higher and was highest of all in unshaded leaves. Evidently weak shading affords a more rapid outflow of assimilates from the leaves. These observations provide an explanation relating the uninterrupted growth of weakly shaded plants to the rapid outflow of assimilates. A significant reduction in starch is found only in the leaves of shaded plants, which also exhibit a particularly intense growth activity.

A number of workers [22-25] have shown that photosynthesis is more rapid when there is an uninterrupted flow of assimilates out of the leaves; they relate photosynthetic activity to accumulation of carbohydrates in leaf tissues.

Shaded plants are darker in color. Colorimetric determination of chlorophyll content shows that it increases (in % of dry wt) as light intensity falls:

Light intensity in thousands of lux	Fourth leaf	Sixth leaf
52.4	1.5	1.4
24.8	3.7	2.1
14.7	2.1	2.0
6.8	7.0	5.8

A comparison of the data on chlorophyll content reveals that it varies in a parallel manner with the degree of shading. The correlation is more often than not significant. At low light intensity the sesame leaf assumes, in the course of its development, the characteristics of leaves of shade plants. A marked difference in the plastid complement is, moreover, observed (Fig. 2). Plastid size is greater in leaves formed in weak light (6.8 thousand lux). The increase in plastid size and chlorophyll content is a manifestation of leaf plasticity and may be regarded as an adaptive reaction to weak light. Our observations are in agreement with the conclusions of Lyubimenko [26], who attaches great significance to the chlorophyll concentration, regarding it as a measure of adaptive response. Gutherie [27] found the greatest chlorophyll content at intensities close to those minimum for the survival of higher plants. Schirley [28] also indicates an increase in chlorophyll with a reduction in light intensity. It is well known that chlorophyll content is closely related to accumulation of dry matter [29]. With a slight reduction of daylight intensity, the latter is increased.

The ecologicophysiological peculiarities of the sesame leaf developing in shade are characteristic not only of sesame. Patterns of adaptation to weak light have been described in the studies of Mukhina [30], Voskresenskaya [31] and others. The absence of a direct relationship between rate of photosynthesis and chlorophyll accumulation is also well documented.

Evidence of adaptive reactions to shade is found in stem structure as well as in leaf structure, being manifested as an intensification of the mesomorphic character (Table 4). Stem diameter and thickness of the secondary xylem decrease in proportion as the light intensity decreases.

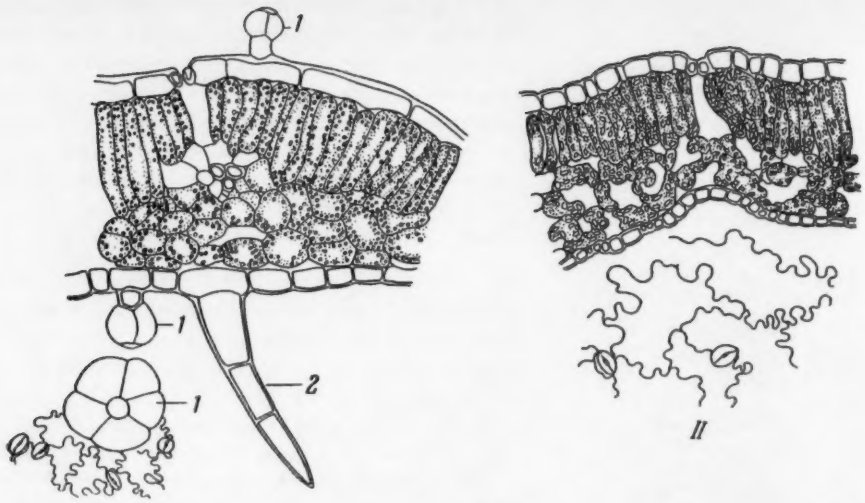

Fig. 2. Cross section of sesame leaves at various light intensities (magnified 280 x). I) 52.4 thousand lux, II) 6.8 thousand lux; 1) glandular and 2) simple hairs.

In our experiments there is a certain variation in fat content of the seeds with changes in light intensity. The greatest difference in fat content is found in plants which are shaded from the beginning of differentiation of the terminal meristem to the formation of pollen tetrads. At this period in the plant's development, the effects of strong light and weak light are profoundly different: at 24.8 thousand lux the oil content of seeds rises 3% and reaches a maximum for the experiment (57%); at 6.8 thousand lux the oil content falls 7.8% to 46.2%, which is the minimum for the experiment. The same treatment (6.8 thousand lux) given after formation of the pollen tetrads, i.e., during the period of formation of floral organs, depresses fat synthesis to a smaller extent — oil content falls to 51.1%. A temporary shading at the time of formation of capsules and seeds, giving 24.8 and 14.7 thousand lux, has the same effect as a permanent shading.

These data relating to oil content and growth, development and fruit formation lead us to the following conclusions: the maximum stimulation of fat synthesis is found in those plants in which there is an activation of growth processes by temporary weak shading at early stages. The effects of permanent and temporary shading on fruit formation are different. Permanent shading depresses fruit formation, the number of capsules being reduced in greater or lesser proportion to the reduction in light intensity (Table 5).

TABLE 4

Changes in Anatomical Features of the Stem of Sesame Plants Grown in Shade (magnification 400 x, in microns, M-50)

Light intensity, in thousands of lux	Epidermis	Collenchyma	Cortex	Central cylinder	Pith
Normal daylight intensity					
52.4	25.1	146.6	83.8	984.65	3226.3
24.8	29.3	146.6	100.5	762.58	3561.5
6.8	25.1	41.9	108.9	301.68	1561.2

An extremely striking example of the promoting effect of temporary shading from the time of formation of flower primordia to the time of formation of pollen tetrads (third group) is illustrated in Fig. 3A. A comparison of the number of capsules and seeds reveals quite definite differences: the greatest number was formed in plants of the third group. The effect of a temporary weak shading as compared with a temporary strong shading is in this case of especial interest, since under both light intensities the number of seeds and their weight turn out to be very similar. Even at 6.8 thousand lux, the number of seeds is 75% of the control. Plant kept in weak or strong shade at this stage of development produced almost the same number of seeds. These experiments led us to the conclusion that weak shading at early stages had not the slightest effect on plant productivity, and even on the contrary, induced a significant increase in oil content of the seeds (3%). A temporary shading at late stages, when floral organs are being formed, has a substantially different effect. Only with a small reduction of light intensity (to 24.8 thousand lux) is a slight (0.7%) increase in the number of seeds obtained (Fig. 3B). At 14.7 thousand lux the yield is severely reduced: the number of seeds fell 42% and the weight 40%. The effect of temporary shading at 6.8 thousand lux at this stage is similar to that of permanent shading, since in both cases fruit formation is virtually absent. A light deficiency during the period of flower formation and also during the period of formation of capsules and seeds alters considerably the conditions of fruit formation. While temporary shading at early stages either increases the number of capsules and seeds or at least leaves it similar to the control, more or less shading at later stages leads to a loss in yield.

Here it should be emphasized that with strong shading the period of formation and maturation of capsules and seeds in the early-maturing variety VNIIMK 78 is prolonged so that this variety approaches late-maturing varieties in the length of its growing period.

TABLE 5

Changes in Certain Economically Valuable Properties of Sesame Resulting from Changes in the Intensity of Daylight (per plant, in % of the control)

Item assessed	Light intensity, in thousands of lux			
	no shading 52.4	24.8	14.7	6.8
No. of capsules	29	45	40.5	7
Wt. of capsules (in g)	8.8	50	32	8
No. of seeds (sp)	1810	37	25	4
Wt. of seeds (in g)	4.8	46	27	4

How can such different adaptive responses to shading be explained? An analysis of data pertaining to the microclimate of the experiment yields an interesting picture. The 24-hour curves for average monthly temperatures and for relative humidity at 24.8 and 6.8 thousand lux are more or less similar for both intensities, both in July and August, but are substantially different from the control. In the night, day and evening hours, the relative humidity is several percent higher than the control in July (Fig. 4A); in August the difference reaches 10%. It should be pointed out that at 24.8 thousand lux the relative humidity is higher at all hours. In the hottest hours of the day, in July, the temperature of the air under the gauze layers is appreciably higher than that of the control, there being no great difference between the temperature under 1 and 4 layers (Fig. 4B). In August, during the afternoon hours the air temperature under one layer of gauze is 1.5-2° higher than that of the control while that under four layers is close to the control. The soil temperature at a depth of 10 cm is markedly lower than that of the control during the hottest hours of the day. In the morning, under 1-4 layers of gauze, it is approximately the same as the control temperature (somewhat higher or lower); at 5 cm depth it is higher under a single layer of gauze; at 10 cm this difference is absent. The moisture content of the soil increases as the degree of shading is increased. At a depth of 90-100 cm there is no effect of shading (Table 6).

In view of the existing information on optimal nitrogen levels for growth and development of each type of plant [32], we determined nitrate content of the soil at various light regimes (Table 6).

The data show that with shading there is a considerable amount of nitrate in the soil. Even under 4 layers of gauze there is very much nitrate, both at flowering (June 30) and at the end of the growing period (September 6). One cannot therefore speak of a nitrate deficiency. The difference in nitrate content associated with experimental treatment may be explained with the help of data on the total weight of the plants. The greatest utilization of nitrates occurs under weak shading with one layer of gauze, in which plants have an especially high rate of growth, a dense foliage and the highest weight; with strong shading (6.8 thousand lux), the weight of the plants is reduced and the utilization of nitrates is minimal. Our observations are in complete agreement

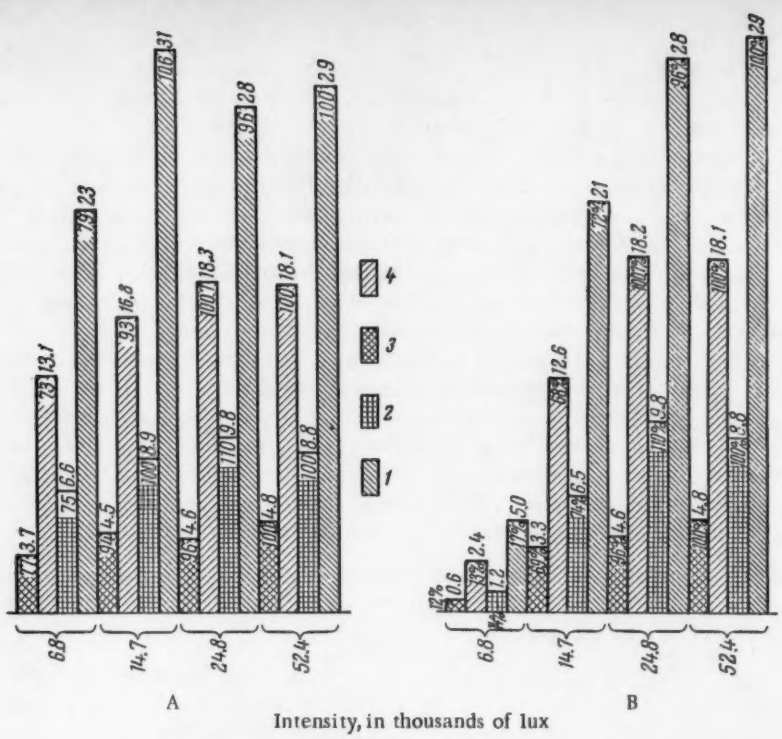

Fig. 3. Effect of temporary shading on certain economically important properties of sesame. A) during the period from differentiation of the terminal meristem to formation of pollen tetrads; B) during the period from formation of pollen tetrads to the first flower (figure inside the bars – percent, outside the bars – absolute); 1) no. of capsules, 2) weight of capsules, 3) weight of seeds, 4) No. of seeds. a) Intensity in thousands of lux.

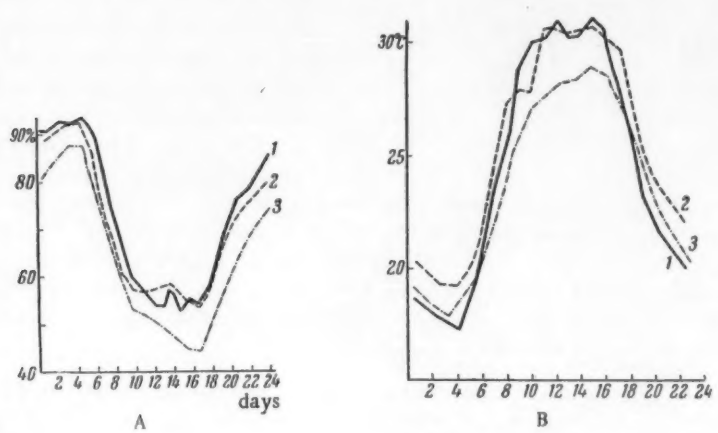

Fig. 4. Average daily curves of relative humidity (A) and air temperature (B). 1) one layer of gauze, 2) four layers of gauze, 3) control.

with the views of Tagmazyan [32] and Chernavskaya [33] to the effect that higher light intensities are associated with a higher level of nitrogen nutrition.

Turning to an analysis of our observations, an extremely important conclusion must first be drawn: at reduced light intensities, from 24.8 down to 6.8 thousand lux, the microclimate is more or less the same: the

relative humidity and the soil moisture content are higher than for normal daylight; the soil temperature is lower during the hottest hours of the day. In the south these are critical factors, since here normal growth and development are to a considerable extent limited by water. Therefore, shading tends to improve the microclimate, exerting a beneficial effect on physiological processes and increasing heat resistance.

TABLE 6

The Effect of Shading on Moisture Content of the Soil and Nitrate Content (June 30)

Depth, in cm	Light intensity, in thousands of lux			
	52,4	24,8	14,7	6,8
	Soil moisture, in %			
0—10	11.3	13	15	14
10—20	17.7	16.7	17.2	19.1
30—40	19.7	21	20	22.1
90—100	19.7	20.1	19.1	21.4
	Nitrate content, in mg			
0—20	25.68	18.23	22.11	55.36
20—40	13.87	5.83	18.16	28.18

Differences in growth, development, fruit formation and plant structure which arise with strong shade may be explained primarily in terms of different light intensities; such differences reflect the influence of light not only as a factor in nutrition, but also as a very important factor directly or indirectly involved in growth and organ formation. Temporary weak shade increases heat resistance even after removal of the shade, and promotes physiological processes (growth, flow of assimilates, chlorophyll synthesis, etc.) and fat synthesis.

SUMMARY

Variations in the intensity of illumination disturb the rhythm of growth and development of sesame. The rate of fructification is more or less proportional to the intensity of light.

Sesame is especially sensitive to illumination immediately following the light stage.

Temporary shading at early phases (beginning with differentiation of the terminal meristem up to the formation of pollen tetrads during gametogenesis) has a beneficial effect on the productivity of the plant. Temporary shading during formation of floral organs, seeds and capsules has an adverse effect on the crop yield and inhibits oil accumulation to the same extent as permanent shading does.

The oil content of seeds decreases with decrease in light intensity. The largest reduction is observed when the plants are shaded at the stage of highest sensitivity to light, i.e., from the time flower primordia are laid down to the formation of pollen tetrads (at gametogenesis): at a light intensity of 28.4 thousand lux the oil content increases by 3% and is maximal; a decrease of illumination to 6.8 thousand lux reduces the oil content by 7.8%.

Temporary shading at this period improves the microclimate, reduces heating of soil, increases its moisture content and leads to an increase in the productivity of the plants and of the oil content of the seeds.

With shading, adaptive phenomena occur which are characteristic of umbraticolous plants and which tend to enhance the mesomorphic character of the plants: leaf thickness, number of stomates, size of stomate apertures, and also stem diameter and thickness of the secondary xylem are reduced in proportion to the reduction in light intensity; a change in the degree of packing of cells of the spongy parenchyma results in a considerable increase in the intercellular volume and an intensification of the mesomorphic character of the leaf; the chlorophyll content increases as does the rate of flow of assimilates from the leaves.

The occurrence of adaptive reactions to shade in sesame, and also the change in productivity and in oil content of the seeds indicates that light intensity has a significant morphogenetic effect.

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CERTAIN ASPECTS OF PHOTOSYNTHESIS IN RELATION TO METABOLISM OF ORGANIC ACIDS AND AMINO ACIDS

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Essentially photosynthesis is the conversion of light energy absorbed by the chlorophyll pigments into chemical energy. This conversion is accompanied by the splitting of water, the hydrogen of which is involved in the reduction of carbonic anhydride. CO_2 is initially fixed in the chloroplasts and phosphoglyceric acid is then formed.

The chief organic substances appearing subsequently are carbohydrates; but they do not constitute the only materials synthesized, since carbonic anhydride (or phosphoglyceric acid) is not necessarily the only substance reduced. Other substances may also be reduced during photosynthesis, nitrates for example. It is therefore not possible to separate in the living organism the mechanism of carbohydrate synthesis from the mechanism of formation or degradation of organic acids and amino acids.

We have studied certain relationships between photosynthesis and the metabolism of organic acids and amino acids in the leaves of the succulent, Bryophyllum daigremontianum Berger (Crassulaceae). The results discussed have been obtained in our laboratory chiefly by Champigny [1-5], Dardart [6] and Jolchine [7, 8].

Plants used in the experiments belonged to a single clone and were grown on inert quartz gravel supplied with a nutrient solution (Fig. 1). The mineral nutrition could thus be precisely regulated.

It is well known that leaves of Crassulaceae become enriched in organic acids during the first 10-12 hours after the beginning of darkness. During this time they fix carbon dioxide. If they remain in darkness for a longer time, these processes are reversed — the leaves lose their organic acids and CO_2 is given off. Under natural conditions, they also lose, during the day, a certain proportion of the organic acids accumulated during the night. Nevertheless, it is possible to induce acid accumulation even under illumination. It is sufficient to saturate the photosynthetic mechanism with carbon dioxide by placing the leaves in an atmosphere with an increased partial pressure of CO_2. The rhythm of nocturnal accumulation and daytime depletion of acids is determined, at least to some extent, by the partial pressure of CO_2, since this rises in the tissues at night as a result of heightened respiratory activity and falls during the day as a result of photosynthetic activity.

The study of the formation and degradation of acids showed, moreover, that these phenomena are directly related to respiratory oxidations and that they are sensitive to the partial pressure of oxygen in the atmosphere. Acid accumulation under anaerobic conditions is therefore insignificant. It is especially rapid during the first 10 hours of darkness when the partial pressure of oxygen is quite high. Subsequently, however, with the prolongation of the dark period, organic acid breakdown is accelerated at higher oxygen levels (Fig. 2). Similarly the fixation of carbon dioxide in the first hours of the dark period and its evolution with the prolongation of the dark period are more rapid the higher the partial pressure of oxygen (Moyse [12, 13]).

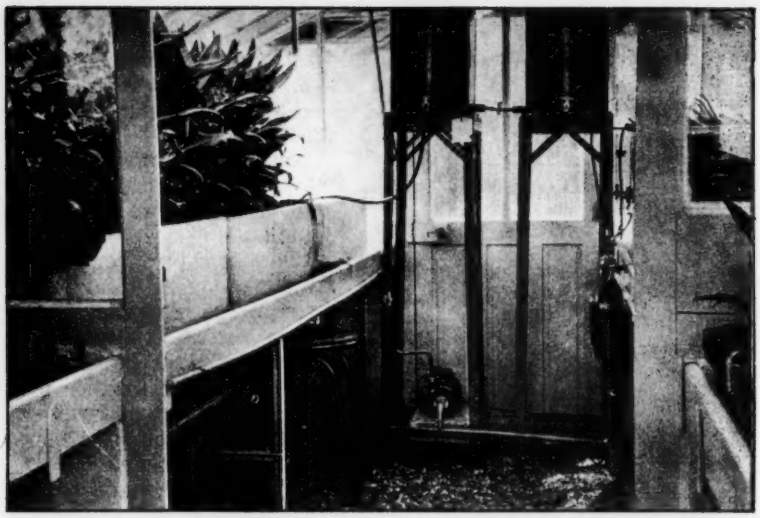
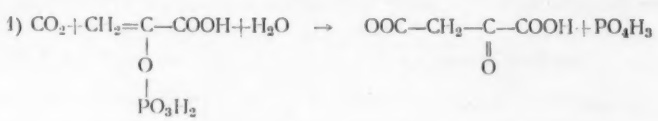
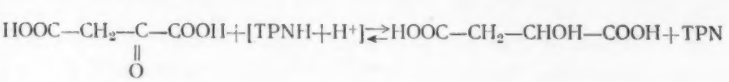


Fig. 1. Culture of Bryophyllum daigremontianum on an artificial nutrient solution. At the left are basins in which Bryophyllum plants are grown. These basins are filled with quartz gravel which is irrigated with a nutrient solution circulating from a reservoir located in the lower part of the center of the photograph. Three times a day the pump, located below the middle of the photography, pumps the solution from the basins back into the reservoir. In this way the rootlets are periodically aerated and supplied with nutrients.

The chief acid accumulated in the dark is malic acid. It is formed by β-carboxylation of phosphoenolpyruvic acid (Walker [14]) followed by reduction of oxalacetic acid. These two reactions may be schematically indicated in the following manner:



1) $CO_2 + CH_2{=}C(OPO_3H_2)-COOH + H_2O \rightarrow OOC-CH_2-C(=O)-COOH + PO_4H_3$



2) $HOOC-CH_2-C(=O)-COOH + [TPNH + H^+] \rightleftarrows HOOC-CH_2-CHOH-COOH + TPN$

TABLE 1

The Effect of Nitrates on $C^{14}O_2$ (number of counts, in thousands per minute, which corresponds to $C^{14}O_2$ fixed by leaves per g dry wt. Partial pressure of CO_2 = 0.045%; specific activity of $C^{14}O_2$ = 19.5 mC per millimole; light = 20,000 lux; t = 20°C)

Exposure time	Leaves taken from plants which continually received KNO_3 (nitrate N = = 5.9 mg per g dry wt)		Leaves taken from plants which were deprived for 48 hr prior to the expts. of KNO_3 (nitrate N < 3 mg per g dry wt)	
	water sol. fraction	insoluble fraction	water sol. fraction	insoluble fraction
Light, 30 sec	14.2	0	58.4	0
Light, 2 min	73.2	0.4	84.4	0.5
Light, 6 min	106.7	39.5	394.1	8.6
Darkness, 1 hr	906.2	2.8	2843	14.4

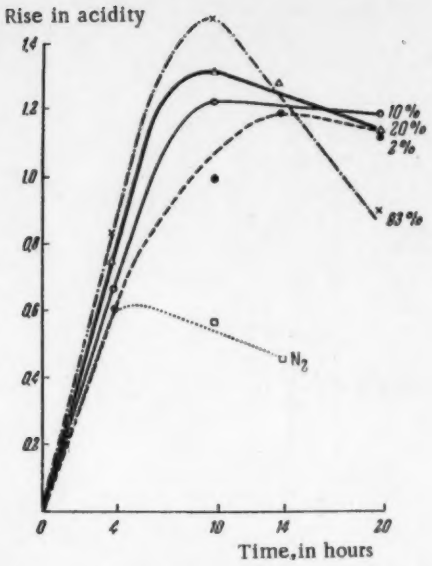


Fig. 2. Increase in acidity, in milliequivalents per gram leaf dry weight, in darkness at a CO_2 partial pressure of 7% and at various partial pressures of oxygen. Oxygen concentration: for N_2, the partial pressure of oxygen is in effect 0.1%. Other partial pressures used were 2%, 10%, 20% and 93%. The figure shows that the increase in acidity during approximately the first 10 hours is more rapid the higher the partial pressure of oxygen. In succeeding hours the decrease in acidity is also more rapid the higher the partial pressure of oxygen.

The first reaction, catalyzed by phosphoenolpyruvate carboxylase, is irreversible, the second, catalyzed by malic dehydrogenase, reversible; with the continuous generation of oxalacetic acid in the first reaction, however, malic acid is accumulated.

The accumulation of other acids in the dark may be observed. Chromatographic analysis of organic acids on silica gel has made it possible to identify acids occurring in leaves (Jolchine [7]).

In practice all the tricarboxylic cycle acids are found, but only malic, citric and isocitric acids are accumulated in quantity. All three are easily oxidized. In a nitrogen atmosphere a slight accumulation of succinic acid may be observed (Moyse and Jolchine [15]). Finally, leaves of Crassulaceae may contain significant amounts of free sedoheptulose. This compound is accumulated principally during prolonged illumination (Dardart [6]).

In recent years we have studied the metabolism of organic acids and amino acids found in Bryophyllum leaves using $C^{14}O_2$. In particular we have investigated the possibility that the carbon of these compounds may be utilized by photosynthesizing leaves in the absence of external CO_2.

Leaves in the same position on the plant and of approximately the same age were exposed to $C^{14}O_2$ in a device illustrated in Fig. 3. After fixation by immersion in boiling ethyl alcohol and extraction of the soluble materials, these were separated on ion exchange resins (cationic and anionic resins). Organic acids were then separated and determined quantitatively on silica gel columns. Amino acids were separated and determined quantitatively by paper chromatography.

TABLE 2

Specific Activity of Malic, Citric, Isocitric and Fumaric Acid Obtained from Leaves after a 6- Minute Exposure to $C^{14}O_2$

Exptl. conditions	Malic acid	Citric acid	Isocitric acid	Fumaric acid
Darkness CO_2 = 0.12%	1327	243	>17	2727
Light, 5000 lux CO_2 = 0.12%	2737	>813	—	—
Light, 20,000 lux CO_2 = 0.10%	6110	>1337	>122	—
Light, 20,000 lux CO_2 = 0.04%	1560	537	—	—

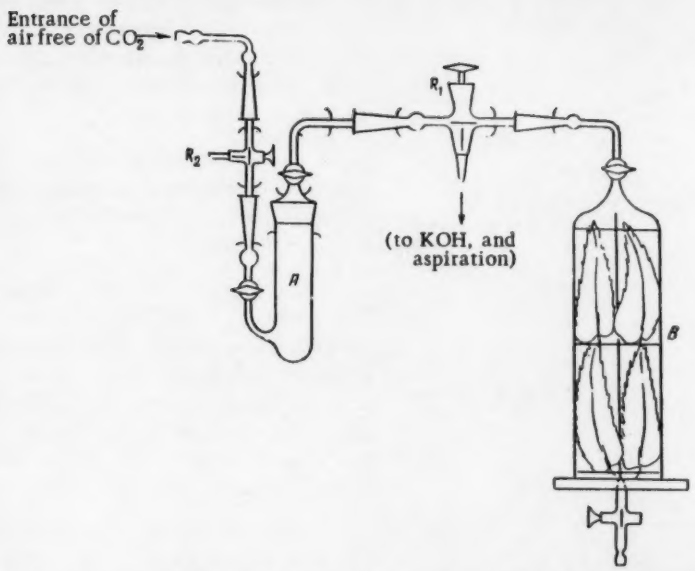


Fig. 3. Apparatus for exposure of leaves of Bryophyllum daigremontianum to an atmosphere containing $C^{14}O_2$. A) vessel containing $BaC^{14}O_3$ as a source of $C^{14}O_2$; B) chamber into which leaves are placed (supported by a metal stage), which is closed below by a metal plate C. Introduction of lactic acid was accomplished with a funnel at the position of the stopcock R_2. The stopcocks R_1 and R_2 made it possible to admit gas to the chamber without admitting outside air and to quickly remove $C^{14}O_2$ from the chamber before opening it. In particular, stopcock R_1 made it possible to draw off air containing $C^{14}O_2$ and to cause it to circulate in KOH absorbing solution.

TABLE 3

Redistribution of C^{14} in Leaves. Radioactivity of the Chief Organic Acids and Amino Acids after Redistribution in the Light (20000 Lux) of Radiocarbon Fixed Previously in the Dark (Upper Part of the Table), and Corresponding Specific Acitivities (Lower Part of the Table)

Exposure time	Malic acid	Citric acid	Isocitric acid	δ-Alanine	Glutamic acid
Thousands of counts per minute per g dry leaves					
Darkness, 1 hr	1981	234	35.3	0.5	18.6
Darkness, 1 hr + light, 30 sec	1838.8	192.5	28.3	2.9	23.7
Darkness, 1 hr + light, 6 min	1739	181	103.9	+	36.6
Counts per minute per mg of each substance					
Darkness, 1 hr	31 700	19 500	720	110	200
Darkness, 1 hr + light, 30 sec	53 390	16 530	1100	360	150
Darkness, 1 hr + light, 6 min	40 440	14 160	3910	—	21 000

With 6-minute illumination (5000 lux), free carbohydrates and phosphorylated compounds as well as amino acids and organic acids were strongly labeled (Fig. 4).

Special analysis of organic acids indicated that in darkness only malic acid is strongly radioactive while in the light during the same length of time fumaric acid and citric acid are also labeled (Fig. 5).

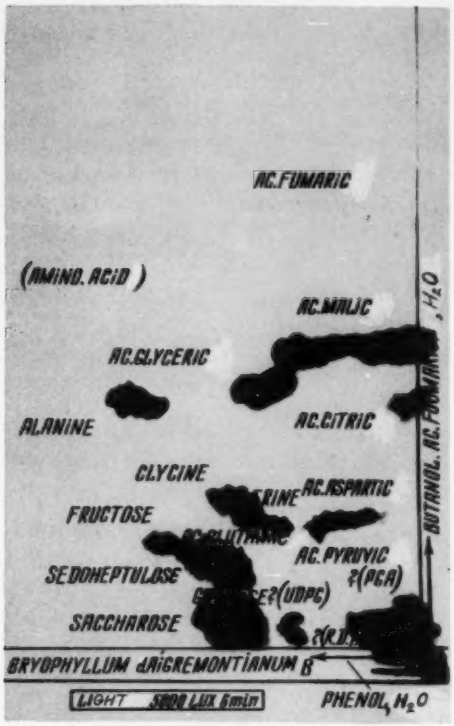


Fig. 4. Radioautograph of an extract of soluble substances from Bryophyllum daigremontianum leaves subjected to a six-minute exposure to light at 5000 lux. Solvent systems: phenol — water, and butanol — formic acid — water. UDPG, PGA, RDP — uridine disphosphate glucose, phosphoglyceric acid, ribulose diphosphate. Note the large malic acid spot.

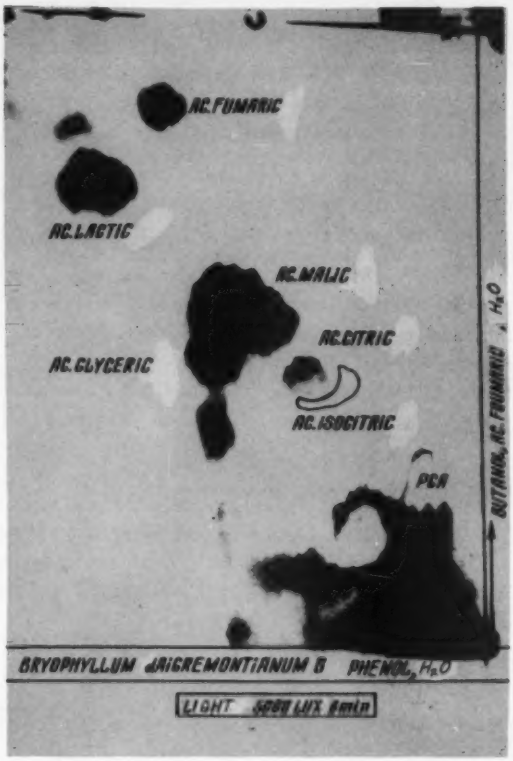


Fig. 5. Radioautograph of the acidic fraction of the soluble substances obtained from Bryophyllum daigremontianum leaves subjected to a six-minute illumination at 5000 lux. Solvent systems: phenol — water, and butanol — formic acid — water. The very prominent spot located at the origin represents phosphorylated compounds. Isocitric acid, located on the chromatogram with bromphenol, is not radioactive. The area where it does not overlap with citric acid is outlined as a crescent. Lactic acid is apparently a derivative of glyceric acid which arises in turn from phosphoglyceric acid.

The following amino acids are labeled during a six-minute exposure in darkness: α-alanine, β-alanine, glycine, serine, glutamic acid and aspartic acid. In light, however, α-alamine and glutamic acid are especially strongly labeled. In addition, leucine is labeled (Fig. 6). The incorporation of radioactivity into various substances during a given exposure time is a measure of the rates of their formation, and is subject to the influence of certain factors, i. e., the nitrate content of leaves or the intensity of illumination.

1. The effect of nitrates was studied in the following manner:

Some of the plants were grown on a nutrient solution containing KNO_3 so that at the time of exposure to $C^{14}O_2$ the leaves contained nitrates. Other plants received no nitrogen for 48 hours prior to collection of leaves for the experiment. Under these conditions the leaves contained little or no nitrogen although externally they

showed no symptoms of nitrogen deficiency. The relative capacities of these two groups of leaves to fix CO_2 are shown in Table 1.

The data in Table 1 show that CO_2 fixation is 3-5 times as slow in leaves containing nitrates as in those depleted of nitrates. The impression is created that in the light CO_2 and NO_3^- compete in the photoreduction reaction. In fact, it is well known that the same hydrogen transfer agent ($TPNH+H^+$) participates in NO_3^- reduction and CO_2 reduction. An analogous competition should take place during dark fixation of CO_2 by β-carboxylation, since the formation of malic acid and the reduction of NO_3^- both require ($TPNH+H^+$). These competitive relations may be illustrated by the following scheme of Champigny [2]:

$$\left.\begin{array}{l}\text{Phosphoglyceric acid}\\ \text{nitrate ion}\\ \text{oxalacetic acid}\end{array}\right\} +TPNH+H^+ \left\{\begin{array}{l}\text{Triose phosphate}\\ \text{ammonium ion (or } NH_2OH)\\ \text{malic acid}\end{array}\right.$$

When leaves are rich in nitrates the amount of $C^{14}O_2$ fixed in amino acids is greater than when they are depleted of nitrates (Champigny [2]). This is in agreement with the observations of Nichiporovich, Andreeva, Voskresenskaya, Nezgovorova and Novitskii [16].

2. The effect of illumination intensity of $C^{14}O_2$ incorporation was shown in two ways: on the one hand the quantity of $C^{14}O_2$ fixed was increased with an increase in intensity, while on the other hand the distribution of radioactive carbon among the various groups of substances was altered with intensity, i. e., at weak intensities the relative content of C^{14} was higher in amino acids and organic acids (Champigny [1]). The increased incorporation of C^{14} into amino acid molecules may be observed only with short exposure periods, since it is rapidly curtailed with the decrease in nitrate content.

At high light intensities, and also with longer exposure periods, the relative content of radioactive carbon fixed in carbohydrates increases.

Nevertheless, light enhances the incorporation of the label into organic acids, and the radioactivity of malic, citric and isocitric acid is therefore increased with an increase in light intensity (Moyse and Jolchine, [17]). The specific activity of these acids is also increased. Inspection of Table 2 shows that at a single partial pressure of CO_2 (0.10-0.12%) the radioactivity of these acids is at least doubled with an increase in light intensity

TABLE 4

Redistribution of Radioactivity in the Light (at 20,000 Lux) after $C^{14}O_2$ Fixation by Leaves in the Dark (S: soluble fraction; I: insoluble fraction)

Exposure time (number of counts)	Percent of soluble fraction			
	organic acids	phosphorylated compounds	amino acids	carbohydrates
Darkness, 1 hr (S: 2843,000; I: 14,400)	96.1	2.7	1.1	0.1
Darkness, 1 hr + light, 30 sec (S: 2,718,800; I: 9,700)	95.6	2.7	1.6	0.1
Darkness, 1 hr + light, 2 min (S: 2,229,400; I: 4700)	98.5	0.6	0.8	0.1
Darkness, 1 hr + light, 6 min (S: 2,708,600; I: 11,000)	95.5	2.6	1.8	0.1

from 5000 to 20,000 lux. At a partial pressure of 0.04% and an intensity of 20,000 lux, the specific activity of malic and citric acid is, nonetheless, higher than in darkness with a CO_2 level three times as high. It thus appears that light induces an acceleration of acid formation, particularly of those oxidative reactions which lead to the formation of citric and isocitric acid.

This was explicitly shown and generalized in a study of the redistribution of C^{14} fixed in the dark during a subsequent period in which the carbon of organic acids was utilized in photosynthesis (Champigny, Jolchine, Moyse [12]).

The experiments were performed in the following manner.

Leaves were kept in an atmosphere containing $C^{14}O_2$ in the dark for one hour. The $C^{14}O_2$ was then removed with a stream of air passed through the chamber containing the leaves (chamber B in Fig. 3). After purification of the atmosphere the leaves were illuminated for 30 seconds, 2 minutes and 6 minutes.

The leaves treated in this way, the unilluminated controls, and the leaves subjected to illumination for the same length of time in the presence of $C^{14}O_2$ were fixed for subsequent analysis of the soluble materials. (Radioactivity of the alcohol-soluble fraction comprises more than 98% of the total radioactivity of the leaves).

A comparison of results summarized in Table 3 shows a rapid increase in the light of total radioactivity and specific radioactivity of isocitric and glutamic acids, while these decrease in citric acid. The effect of light on glutamic acid formation is still more interesting in view of the fact that this acid is the main point of incorporation of mineral nitrogen into proteins. With respect to malic acid, the slow decrease in its total radioactivity is accompanied by an increase in its specific activity. These two phenomena are related in that there is a simultaneous decrease in the total amount of malic acid as a result of its decarboxylation in the cytoplasm and an increase in the proportion of labeled carbon as a result of fixation by carboxylation which proceeds, most likely, in the chloroplasts.

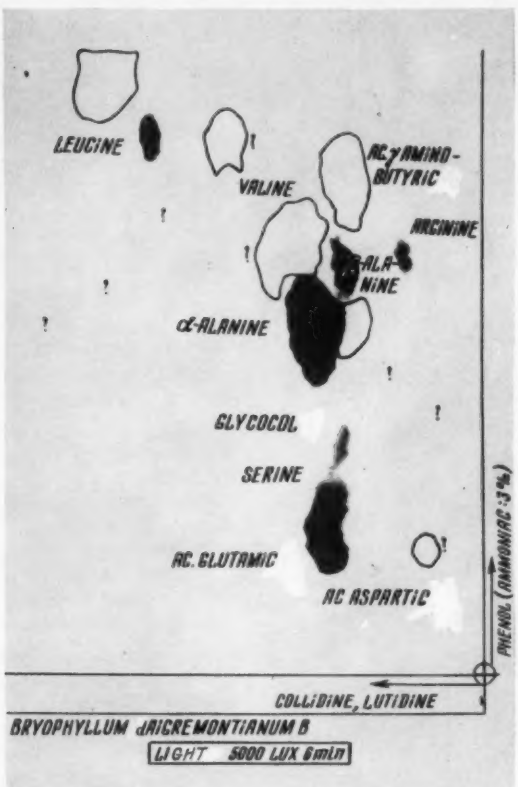


Fig. 6. Radioautograph of the amino acid fraction from Bryophyllum daogremontianum leaves subjected to a six-minute illumination at 5000 lux. Solvent systems: collidine – lutidine, and ammonia – phenol. Nonradioactive acids, located with ninhydrin, are also indicated.

At present we are carrying on investigations to localize these two opposing, simultaneously occurring processes. Finally, with respect to alanine, the small increase in its radioactivity and specific activity under illumination indicates an enhanced formation in light.

The first of these results requires a certain elucidation. Working with Chlorella, with Scenedesmus, and with barley leaves, Benson and Calvin [19], Calvin and Massini [20], and Bassham, Shibata, Steinberg, Bourdon and Calvin [21] showed that in light the radioactivity of citric and isocitric acid, as well as that of glutamic acid, was lower than in the dark, with the same exposure time. From this they concluded that oxidative processes are slower in the light as a result of blocking of reduced thioctic acid, which furnishes the reducing power for photosynthesis. This acid therefore cannot participate in the splitting of pyruvic acid, and this in turn leads to an inhibition of the tricarboxylic acid cycle.

Actually, however, it cannot be said that light hinders oxidative processes in cells, taken as a whole. Oxygen absorption is not slowed down during illumination (Brown [22]). It is possible, though, that oxidation of carbohydrates by the glycolytic pathway or the tricarboxylic acid cycle is slowed down in the chloroplasts. This could take place as a result of inhibition of phosporylation which leads to the formation of hexose diphosphates through hexose monophosphate. Such an inhibition was shown by Engel'gardt and Sakov [23].

This enables one to understand how there may be a partial inhibition by light of carbohydrate oxidation; a formation of oxidized compounds just after the photolysis of water would preclude the evolution of oxygen.

TABLE 5

Distribution of Radioactivity in the Light among Various Compounds from Leaves with Adventitious Roots after Absorption of Glutamic Acid Labeled with C^{14} in Position 3-4 or 1 (duration of experiment, 6 hr; T = 21°C; light intensity, 20,000 lux)

Fractions and amino acids	Glutamic acid labeled in position 3-4		Glutamic acid labeled in position 1	
	Counts per minute per g dry leaves			
Insoluble fraction	8,500		Traces	
Soluble fraction	19,300		1,100	
	Distribution of activity in various soluble fractions			
Aspartic acid	0.6		3.2	
Glutamic acid	45.0	Amino acids	75.4	
α-Alanine	0.5	76.6	0	
Glutamine	23.2		7.4	92
α-Aminobutyric acid	5.2		0	
Leucine	1.5		0	
Pyrrolidine and carbonic acid	Traces		6.0	
Pyruvic acid	11.0		1.2	
α-Ketoglutaric acid			0.3	
Succinic acid	2.4		0.6	
Fumaric acid				
Glycolic acid				
Aconitic acid	1.5	Organic acids	0.2	3.7
Malic acid	2.0	23.4	Traces	
Citric acid	3.0		0.3	
Isocitric acid	1.2			
Carbohydrates	Traces		4.3	

TABLE 6

Distribution of C^{14} (in %) in the Malic Acid Molecule after Fixation of $C^{14}O_2$ by Leaves

Position of carbon	Darkness, 1 hr	Light, 2 min	Light, 6 min	Darkness 1 hr+ light, 30 sec*	Darkness 1 hr+ light, 2 min*	Darkness 1 hr+ light, 6 min*	Darkness 1 hr+ light 15 min**
4 — COOH	70	50	43	65	66	70	71
3 — CH_2	0	16	+	0	0	0	—
2 — CHOH							
1 — COOH	30—32	34	35	34	29	29	32

*$C^{14}O_2$ removed prior to illumination

**Illumination in presence of $C^{14}O_2$.

In contrast, oxidation of organic acids, especially of malic acid in the cytoplasm, is hastened by light just as it is hastened by high partial pressures of oxygen in the atmosphere (Moyse [24]). Thus, it is possible that promotion of formation of isocitric and glutamic acid in the light is due to an acceleration of oxidative processes involving oxygen of photosynthetic origin.

Photosynthetic redistribution of radioactivity under the influence of light is still a slow process, however (Table 4).

Photosynthesis proceeding in the presence of $C^{14}O_2$ insures a rapid synthesis of phosphorylated compounds, amino acids and carbohydrates, while at the same time the relative amount of label in organic acids declines with time. In contrast to this, photosynthesis proceeding in the presence of labeled organic acids or their derivatives as the source of carbon is very slow, and this leads in turn to an extremely slow formation of labeled phosphorylated compounds, amino acids and carbohydrates.

A similar situation was found by Milhaud, Benson and Calvin [25] in connection with the utilization by Scenedesmus cells of carbon of pyruvic and hydroxypyruvic acid, and by Kursanov and Kryukova [26] in connection with the utilization by Phaseolus vulgaris leaves of carbon from the Krebs cycle acids in photosynthesis.

A circumstance so disadvantageous as this is due primarily to the necessity of first oxidizing organic acids, since their carbon may be utilized in photosynthesis only after its release in the form of CO_2.

The direct utilization of even such a small molecule as pyruvic acid is not possible (as will be shown below).

The first proof of the hypothesis was obtained with Bryophyllum leaves to which were supplied glutamic acid labeled with C^{14} in either position 1: (HOOC$-CH_2-CH_2-$CH(NH$_2$)$-C^{14}$OOH), or in position 3-4: (HOOC$-C^{14}H_2-C^{14}H_2-$CH(NH$_2$)$-$COOH) (Champigny [3]).

Leaves used in the experiments possessed adventitious roots. The roots were immersed in solutions of glutamic acid labeled in either of the two positions. Radioactivity of a solution was equal to 10 mC. The results presented in Table 5 were obtained after 6 hours in the light.

Table 5 shows that part of the carbon from the absorbed glutamic acid is distributed among various amino acids. It should first be pointed out that α-aminobutyric acid becomes radioactive only after absorption of glutamic acid labeled in position 3-4. It contains no radioactivity when the absorbed glutamic acid is labeled in position 1. This confirms the formation of α-aminobutyric acid by decarboxylation of glutamic acid at position 1(Champigny and Lioret [27]).

The other part of the C^{14} is found in the organic acids; it is proportionately greater when the label in glutamic acid is in position 3-4. This indicates an oxidation of α-ketoglutaric acid arising from glutamic acid by deamination. Decarboxylation of α-ketoglutaric acid leads to the formation of the four-carbon acids, and decarboxylation of malic acid leads to the formation of pyruvic acid. The acids arising in this manner contain a significant proportion of the radioactivity only if the label in glutamic acid is at position 3-4.

Under these conditions there is almost no radioactivity in carbohydrates, however. When the label is at position 1, on the other hand, there is considerable radioactivity in this fraction; this may be explained by the release of $C^{14}O_2$ by decarboxylation of glutarate and the formation of γ-aminobutyric acid. From this it follows that pyruvic acid may not be directly utilized in the synthesis of carbohydrates in the presence of light. In other words, the reaction, phosphoglyceric acid → pyruvate+H_3PO_4, proceeds in vivo only from left to right.

Finally, it seemed of interest to study the distribution of radioactive carbon fixed in malic acid in the light and in the dark. Malic acid exhibits, on the one hand, the most marked increase in radioactivity in the dark, while on the other hand it shows the largest amount of carbon redistribution in the light. In every case it is altered to a much greater extent than are the other acids.

The experiments were performed by Jolchine [8]. Carbon 4 was removed with malic decarboxylase from Lactobacillus arabinosus (strain 17-5; decarboxylation of carbon 1 was accomplished with H_2SO_4 at 55-65° for $2\frac{1}{2}$ hours).

The CO_2 was collected as $BaCO_3$, after which its radioactivity was measured. It was possible to calculate the radioactivity of the second and third carbon atoms (Table 6).

After fixation of $C^{14}O_2$ in darkness, the ratio of radioactivity of carbon 1 to radioactivity of carbon 4 was 2:1. A subsequent illumination induced only a very slow redistribution. The change was so slight that even after a 15-minute exposure to light it could not be detected. With short-term illuminations in the presence of $C^{14}O_2$, the distribution is approximately the same, although radioactivity appears quite rapidly in positions 2 and 3 also. These results are in agreement with results obtained by Bradbeer, Ranson and Stiller [28].

The original ratio of radioactivity between carbons 4 and 1, 2:1, which is obtained under illumination, is explained by the fact that phosphoenolpyruvate is a derivative of phosphoglyceric acid. This acid is formed by the carboxylation of ribulose diphosphate. Two molecules of phosphoglyceric acid are formed of which only one is labeled in position 1:

$$RuDP + C^{14}O_2 \rightarrow PGA + PGA^{*1}$$

In this manner equal numbers of molecules labeled at carbon 1 and of unlabeled molecules arise. Carboxylation at position 4 introduces an equal amount of radioactivity into every phosphoenolpyruvate molecule, and as a result half the malic acid molecules possess a label at positions 1 and 4, and half are labeled only at position 4. Subsequently, reactions of the oxidative cycle as well as the photosynthetic cycle alter this distribution and radioactivity appears in the second and third atoms as well.

In darkness a similar distribution between the first and fourth carbon atoms may be obtained in the same way if phosphoenolpyruvate is formed by carboxylation of ribulose diphosphate, which can be in turn formed by the oxidation (with decarboxylation) of gluconic acid (the so-called phosphopentose path of glucose oxidation, cf. Lioret [29]).

The conversion of sedoheptulose is another possible path of formation of the necessary ribulose diphosphate.

This might take place if after a period of illumination during which sedoheptulose is accumulated (Dardart [6]), there is a dark period. The amount of sedoheptulose falls markedly in the dark, probably as a result of conversion into ribulose phosphate. This can be checked by comparison with the amount of ribulose diphosphate appearing in the light. The process may be schematically indicated in the following way: sedoheptulose phosphate+triose phosphate→ribulose phosphate (cf. Calvin [30]).

Sedoheptulose may thus serve as a potential source of pentoses freely utilized in the dark.

The distribution of radioactivity in malic acid formed in the dark is such that with redistribution in the light accompanied by decarboxylation of carbon atom four, $C^{14}O_2$ and pyruvic acid, certain molecules of which are labeled in position 1, are formed.

The slow incorporation of $C^{14}O_2$ into carbohydrates under these conditions indicates once more that pyruvic acid as such does not participate in photosynthesis.

SUMMARY

1. It is shown that photosynthesis is not concerned solely with carbohydrate synthesis. Depending on conditions of illumination and plant nutrition, other materials may also be synthesized in greater or less quantity. These factors are associated with various patterns of distribution of reduced carbon among carbohydrates, organic acids and amino acids.

2. In the presence of nitrates, there is competition between them and CO_2 in the assimilating tissue for photosynthetic reducing power. This leads to a more rapid synthesis of amino acids.

3. At weak light intensities amino acid synthesis is relatively more rapid. At stronger intensities, carbohydrate synthesis becomes more significant. This emphasizes the direct involvement of light in protein synthesis.

4. Fixation of $C^{14}O_2$ by β-carboxylation of phosphoenolpyruvate is possible both in light and in darkness, but it is insignificant in the former case inasmuch as the bulk of the three-carbon compounds and also of CO_2 is channeled into carbohydrate syntheses. At weak intensities, however, a significant portion of the available carbon dioxide is fixed by β-carboxylation.

5. Oxidation of organic acids is more rapid in the light. The acceleration is clearly manifested, on the one hand, by a decrease in malic acid content of the leaves, and on the other hand by an increase in the

radioactivity and specific activity of isocitric and glutamic acid. This increase in oxidative activity may take place in the cytoplasm as a result of an increased partial pressure of oxygen in the tissues.

6. Redistribution in the light of radioactive carbon incorporated into malic acid in a preceding dark period shows that organic acids, even pyruvic acid, are not directly utilized in photosynthesis. The distribution of radioactive carbon introduced in the form of glutamic acid indicates the same thing. This evidence all points to the necessity of decarboxylation or extensive oxidation of organic compounds before their carbon is available for photosynthesis. Probably only CO_2 can be directly utilized.

7. The distribution of $C^{14}O_2$ incorporated into malic acid in both the light and the dark emphasizes the leading role which pentoses must play in various mechanisms of CO_2 fixation.

8. These results show how difficult or even impossible it is to draw a sharp line between the phenomenon of photosynthesis (formation of carbohydrates) and other metabolic processes.

Each experiment performed has given rise to new questions, although it is considered that some problems have been solved. It may be that the most valuable result of our work resides in this fact.

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GLYCOLYTIC ENZYMES OF THE CONDUCTING TISSUES OF SUGAR BEET

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At the present time, when the over-all level of knowledge of plant and animal metabolism has been significantly raised and methods of investigation have been more and more refined, it is becoming possible to study in greater detail the metabolism of tissues, specialized as to function, which have heretofore been studied anatomically for the most part and whose functions have been deduced on the basis of such studies.

One of the tissue systems extremely characteristic of higher plants, whose development has been linked with the evolution of flowering plants, is the conducting tissue system.

A study of the biochemical characteristics of conducting tissues would be of direct value in the elucidation of the mechanism of translocation, which as yet has not been fully explained.

The purpose of this study was to examine more closely the enzymes of the conducting tissues. It was devoted to the fibrovascular bundles of sugar beet.

It has been shown in this laboratory [1, 2, 3] that the fibrovascular bundles of sugar beet petioles have an extremely intense respiration. This is not restricted to sugar beet, since it has been observed in other plant species [1, 4, 5, 6].

It has also been shown that increased respiratory activity is characteristic of both phloem elements and living xylem elements [5], which indicates on the whole a rapid metabolism of each specialized tissue system. The intensive respiration of the phloem, one of the arguments in favor of the hypothesis of the metabolic nature of translocation, even though it is only an indirect argument, deserves serious consideration. It was not the purpose of this study to investigate directly the mechanism of translocation in plants; it seemed appropriate, however, to further investigate the previously observed fact of an intense respiratory activity in the fibrovascular bundles by examining in detail the enzymes peculiar to such tissues.

There is a certain amount of published data on this subject, but these data are far from complete, and are, to a certain degree, moreover, contradictory. Kursanov and Turkina [7] found in conducting tissues of sugar beet invertase, phosphatase, phosphorylase, and cytochrome oxidase [3]. The presence of cytochrome oxidase was confirmed by Willenbrink [4] and Ziegler [5]. This is of special interest since this enzyme represents the final step in the respiratory chain. The observation by Ziegler [5] of a very active succinic dehydrogenase in the vascular bundles of Heracleum mantegazzianum, which indicates, apparently, that the tricarboxylic acid cycle functions in these tissues is of basic importance. The work of Wanner [8], in which he found in the phloem exudate of Robinia pseudoacacia phosphatase, apyrase and phosphoglucomutase, but failed to find such important glycolytic enzymes as hexokinase, phosphohexoisomerase and aldolase, is well known. The special feature of Wanner's experiments consisted in the fact that he determined enzymes in sap exuded by the sieve cells. It might be for this reason, that he did not find enzymes which are bound to the cytoplasm. Moreover, it might be assumed that enzymes found in phloem exudate represent that part of the total enzyme complement which at that time, for some reason, is excluded from direct participation in metabolic processes, and therefore such enzymes would not be characteristic of the metabolism of the cells excreting them. In view of this we determined enzymes in fibrovascular bundles taken in their entirety. Naturally we were dealing with an extremely heterogeneous tissue of which the phloem comprised but a small part. We were concerned, however, to characterize the fibrovascular bundles as a conducting tissue system and were not interested in applying the results to the

interpretation of the mechanism of phloem function. In order to obtaine some idea of the relative activity of the enzymes in these tissues, we made most of the determinations in parallel in the vascular bundles and the parenchymatous tissues surrounding them.

In this study we endeavored to learn more about the glycolytic enzymes in the fibrovascular bundles and the parenchyma of sugar beet petioles, for which we made a detailed study of hexokinase, apyrase, phosphoglucomutase, phosphohexoisomerase and aldolase.

EXPERIMENTAL PART

Hexokinase

The hexokinase reaction consists in the phosphorylation of hexose by ATP. This reaction has been studied in detail in animals, yeast, bacteria, and in a number of plants. Yeast hexokinase has been isolated in crystalline form [9]. It has been found by a number of workers in muscle, liver, brain, and other animal organs [10-18]. Its reversible character has recently been shown [14, 15]. To those who are interested in translocation in plants, the work of Sols [16], who in the course of an investigation of the hexokinase of intestinal mucosa concluded that it does not participate in the absorption of sugars from the intestine, is of interest. In his opinion, phosphorylation is not, in general, part of the absorption process. The role of hexokinase of the mucosa, according to him, is to initiate glycolysis necessary to the tissue itself.

In comparison with the intensive study of hexokinase from animal tissues and yeast, there has been little investigation of this enzyme in higher plants. Bonner has shown [17], however, that sap from oat coleoptiles can phosphorylate glucose with the formation of fructose 1,6-diphosphate. Kotel'nikova [18] found hexokinase in potato. Bonner and Millerd [19] found it in mitochondria isolated from Phaseolus aureus seedlings. It has also been found by Saltman [20] in a number of other plant tissues. Recently Medina and Sols [21] isolated a specific fructokinase from peas which phosphorylates fructose. Finally, Wanner [8] looked for it in the phloem exudate of Robinia pseudoacacia but did not find it.

In our experiments hexokinase activity of the vascular bundles and parenchyma (from which the bundles had been removed) of sugar-beet petioles was determined by the ability of enzyme preparations to form glucose-6-phosphate from glucose and ATP. Enzyme preparations were made in the following manner: the plant material was fixed in liqued nitrogen and carefully ground in a frozen condition. The ground tissue was used immediately or lyophilized and stored in the cold. Activity of the lyophilized preparations was essentially the same as that of preparations from fresh tissues even after storage for several months. To obtain preparations from freshly ground tissues, the latter were extracted for 20 minutes in an equal volume of 0.14 M veronal buffer, pH 7.0, at 2-4°. The homogenates were passed through cotton cloth, and the extract was centrifuged 4 minutes at 3000g. The supernate was used without further treatment.

In the case of lyophilized preparations, the dry material was extracted 3 hours at 2-4° with ten volumes of 0.14 M veronal buffer or 0.05 M tris buffer [tris (oxymethyl) aminomethane]. The homogenates were passed through cloth and centrifuged 4 minutes at 300g. Whether fresh or lyophilized tissues were used, an amount of preparation corresponding to 10 grams of tissue dry weight was always used. These extracts always contained a significant amount of sugar and inorganic phosphorus (0.06-0.085 M monosaccharides and about 0.1 M sucrose in extracts from the conducting tissues and 0.085-0.1 M monosaccharides from the parenchyma).

The experiments were therefore performed without the addition to the reaction mixture of glucose (or fructose) and inorganic phosphorus. Usually a reaction mixture contained: 0.014 M ATP, 0.014 M $MgSO_4$, 0.05 M NaF, 4 ml 0.14 M veronal or 0.05 M tris buffer and 12 ml tissue extract. The total reaction volume was 16 ml, the pH 7.2. The experiments were run 30 minutes at 36°. The enzyme was inactivated by 5% trichloracetic acid.

It was shown in special experiments that the greatest quantity of hexose monophosphate is formed during incubation of the tissue extract with ATP at pH 7.2 in the first 30 minutes.

The high level of hexoses and inorganic phosphorus in the extracts made the determination of activity by decrease in glucose or in easily hydrolyzable phosphate of ATP plus inorganic phosphate, i. e., by the methods used by other workers studying plant hexokinase or fructokinase [18, 20, 21], difficult.

We therefore determined hexokinase activity directly by measuring the amount of glucose-6-phosphate (and fructose-6-phosphate) formed; these products were separated by quantitative paper chromatography. Both

TABLE 1

Hexokinase Activity in the Vascular Bundles and Parenchyma of Sugar-Beet Petioles

Tissue	Time, min	Glucose-6-phosphate	Fructose-6-phosphate	Increase in 30 min		
				glucose-6-phosphate	fructose-6-phosphate	total esters
In mg of hexosemonophosphate per 10 g dry wt						
Conducting tissues	0	0.49	0.11	–	–	–
	30	1.99	0.36	1.50	0.25	1.75
Parenchyma	0	0.13	0.07	–	–	–
	30	0.54	0.09	0.41	0.02	0.43
In mg of hexosemonophosphate per 100 mg protein nitrogen						
Conducting tissues	0	1.79	0.39	–	–	–
	30	7.24	1.31	5.45	0.92	6.37
Parenchyma	0	1.56	0.77	–	–	–
	30	6.23	1.05	4.67	0.28	4.95

experimental and control samples were fractionated initially by the method of Umbreit [22], with the production of a second fraction containing the Ba salts of hexosemonophosphates, which are insoluble in alcohol. Barium was precipitated three times in the form of $BaSO_4$, and the fraction was then taken to a small volume. The solution was then spotted on paper and glucose-6-phosphate and fructose-6-phosphate were separated in the form of their boric complexes using the following solvent system: methanol (12) – 25% ammonia (2) – 3% boric acid (3). Separation was continued for 24 hours at 20° on a one-dimensional descending chromatogram. "Slow" chromatographic paper from the Volodar No. 2 plant in Leningrad was used. Prior to use the paper was washed with 20% formic acid and then with a solution of 1% acid in 60% alcohol [23]. The hexosemonophosphates were clearly separated in this manner and the spots were subsequently eluted with water. The sugar moieties were determined colorimetrically with anthrone [24]. The position of the hexosemonophosphate spots was determined by examination of chromatograms under Bromberg's ultrachemiscope (determination of the position of AMP) and by reaction with the Hanes and Isherwood reagent, applied as supplementary (control) streaks.

Results of a single determination of hexokinase in the vascular bundles and parenchyma of sugar-beet petioles are presented in Table 1. These results clearly show that vascular bundles and also parenchymatous tissues of petioles contain this enzyme.

The table also shows that usually fructose-6-phosphate is formed together with glucose-6-phosphate in the reaction mixtures, although to a markedly smaller extent (15-20% for fructose-6-phosphate and 80-85% for glucose-6-phosphate).

This may be due to the action of a specific fructokinase or to the isomerizing action of phosphohexoisomerase, the presence of which, in conducting tissues of sugar-beet, we have previously shown [25].

From Table 1 it can be seen that the conducting tissues possess a more active hexokinase than the surrounding tissues. The rate of phosphorylation of glucose by extracts of conducting tissues was 3.7 times that of extracts from parenchyma (based on 10 g dry tissue wt). Results were also calculated on a protein nitrogen basis in order to obtain an idea of the intensity of phosphorylation in the protoplasm itself. Results of such a calculation showed that even in this case hexokinase activity in the conducting tissues exceeds that in the parenchyma, though not to such a great extent.

These experiments show, thus, that conducting tissues of sugar beet possess a marked capacity to activate hexose by phosphorylating it, which may serve to initiate glycolysis and the subsequent respiration [1, 2, 26]. Besides this, the presence of phosphorylating enzymes may also lead to the synthesis of specific polysaccharides which are abundant in vascular tissues, but whose nature is still unclear.

Our data also showed that hexokinase activity in the petiole parenchyma, a tissue which evidently has no specialized function, was lower then in conducting tissues.

TABLE 2

Hexokinase Activity of the Conducting Tissues and Parenchyma of Sugar-Beet Petioles at Various Periods of Plant Growth (in mg of hexosemonophosphate per 10 g dry wt, 1958)

Tissues	Time, min	July 8				July 30				August 20				September 25			
		Glucose-6-phosphate	Increase in 30 min: Glucose-6-phosphate	Increase in 30 min: Fructose-6-phosphate	Increase in 30 min: Total esters	Glucose-6-phosphate	Fructose-6-phosphate	Increase in 30 min: Glucose-6-phosphate	Increase in 30 min: Fructose-6-phosphate	Increase in 30 min: Total esters	Glucose-6-phosphate	Fructose-6-phosphate	Increase in 30 min: Glucose-6-phosphate	Increase in 30 min: Fructose-6-phosphate	Increase in 30 min: Total esters	Glucose-6-phosphate	Fructose-6-phosphate	Increase in 30 min: Glucose-6-phosphate	Increase in 30 min: Fructose-6-phosphate	Increase in 30 min: Total esters
Parenchyma	0	0.40	—	—	—	0.34	0.15	—	—	—	0.44	0.15	—	—	—	0.63	0.23	—	—	—
	30	2.02	1.62	0.11	1.73	1.61	0.38	1.27	0.23	1.5	2.53	0.52	2.09	0.37	2.46	2.30	0.54	1.67	0.31	1.93
Conducting tissues	0	0.18	—	—	—	0.17	0.06	—	—	—	0.24	0.09	—	—	—	0.23	0.08	—	—	—
	30	0.63	0.45	0.08	0.53	0 57	0.14	0.40	0.08	0.48	0.65	0.17	0.41	0.08	0.49	0.88	0.24	0.65	0.16	0.81

If translocation of organic materials is related to the metabolism of the conducting system, then it might be expected that at different times in the growth period, in relation to the age of the plant and the accumulation of sugar reserves, the hexokinase activity in the vascular bundles would fluctuate in accordance with the intensity of translocation. On this assumption we designed experiments to determine hexokinase activity of the conducting tissues and the parenchyma of sugar beet petioles during the growth period from the appearance of the first developed leaves to the cessation of sugar accumulation.

Results of these experiments are presented in Table 2.

They show that hexokinase activity in the fibrovascular bundles fluctuated during the growing period. The period of greatest activity coincided with the period of rapid transport of sugars from the leaves to the root (August). Fructose-6-phosphate was formed in the conducting tissues together with glucose-6-phosphate throughout the entire growing period. As in previous experiments (see Table 1), the amount of fructose-6-phosphate was not large (6-15% of the total hexosemonophosphate synthesized).

Hexokinase activity of the parenchyma is almost unchanged during the growing period, and only at the end of the season, when the petioles are evidently beginning to serve as storage depots, does it increase slightly. The ratio of glucose-6-phosphate formed to fructose-6-phosphate increases markedly at this time.

Results of Table 2 confirm the earlier observed fact that hexokinase activity of the fibrovascular bundles is higher than that of the parenchymatous tissues (2,6-5 times). The greatest difference in activity is encountered in the second half of August, i.e., in the period of rapid transport of sugars to the root. At this time the rate of phosphorylation in the conducting tissues is five times that in the parenchyma.

Table 2 also indicates the amounts of individual hexosemonophosphates contained in the different tissues. It is shown that glucose-6-phosphate predominates in both the vascular bundles and the parenchyma; there is approximately twice as much in the bundles as in the parenchyma. In a chromatographic analysis of the phosphate esters of the second fraction (according to Umbreit), unknown phosphorus compounds were found; however,

none of these was identified as ribose-5-phosphate or sedoheptulose-7-phosphate, compounds characteristic of the oxidative degradation of glucose.

Apyrase

We have found another enzyme participating in conversions of ATP in both the vascular bundles and the parenchyma of sugar beet petioles; this enzyme, apyrase, degrades the high-energy bonds of ATP. Its activity was determined by the rate at which labile phosphate groups of ATP are split off by tissue extracts in the presence of $CaCl_2$ and veronal buffer, pH 6.2. These determinations indicated a somewhat higher activity in the conducting tissues, 280 μg of phosphorus per gram dry wt of tissue split off in 15 minutes as compared with 200 μg of phosphorus per gram dry wt of tissue for the parenchyma. The total apyrase activity of the conducting tissues and of the petiole as a whole was extremely small in comparison with the activity of this enzyme in potato tubers.

According to the data of Kotel'nikova and Solomatina [27], apyrase of tubers splits off 8000-12000 μg of phosphorus per gram dry wt in 15 minutes.

Apyrase was also found in phloem exudate of Robinia pseudoacacia by Wanner [8]; because of the difference in experimental design, however, it is difficult to compare his data with ours.

Phosphoglucomutase

In the study of enzymes of the vascular bundles of sugar beet which participate in sugar conversions, the demonstration of phosphoglucomutase, which reversibly converts glucose-6-phosphate into glucose-1-phosphate, might prove to be of extreme interest.

There is a report of phosphoglucomutase in beet: in the leaf plastids and the roots [28]; its activity is, however, very low according to the published data.

We determined phosphoglucomutase activity by measuring the decrease in acid-labile phosphorus of glucose-1-phosphate at various time intervals (from 30 minutes to 120 minutes) in a reaction mixture containing tissue extract, NaF and $MgSO_4$; pH of the reaction mixture was 7.0.

Even with 120 minutes exposure, we were unable to find any decrease in acid-labile phosphorus or accumulation of glucose-6-phosphate. Thus, no evidence for phosphoglucomutase in the conducting tissues or in the petioles as a whole was found. These data are not in accord with those of Wanner [8], who found that this enzyme is the only glycolytic enzyme in phloem exudate of Robinia pseudoacacia. This may be due to the difference in experimental material.

Phosphohexoisomerase

The interconversion of glucose and fructose in the fibrovascular tissues of sugar beet previously observed by us [25, 29] was an indirect indication of the presence of phosphohexoisomerase. In this study the enzyme's activity was determined directly according to the quantity of fructose-6-phosphate formed from glucose-6-phosphate in the presence of tissue extracts.

Extracts prepared from lyophilized tissues by the method described above served as the enzyme source (see determination of hexokinase activity). An amount of extract which corresponded to 5 grams tissue dry weight was used.

The reaction mixture included the following compounds: 0.04 M glucose-6-phosphate, 0.047 M NaF, 0.14 M veronal buffer in 1 ml, 5 ml tissue extract. Total volume was 6 ml, pH, 7.5. The reaction proceeded for 20 minutes at 37°. The enzyme was inactivated by the addition of 5% trichloracetic acid and the reaction mixture was subsequently fractionated according to Umbreit's method [22]. The second alcohol-soluble fraction containing glucose-6-phosphate and fructose-6-phosphate was then analyzed. After three precipitations of this fraction the fructose moiety of the fructose-6-phosphate was determined according to the method of Roe.

The results of a determination of phosphhexoisomerase activity are presented in Table 3.

TABLE 3

Activity of Phosphohexoisomerase in the Fibrovascular Bundles and Parenchyma of Sugar-Beet Petioles (in mg of fructose-6-phosphate per 10 g tissue dry wt and per 100 mg protein nitrogen)

Tissue	Time, min	Fructose-6 phosphate, in mg per 10 g tissue dry wt	Increase in fructose-6-phosphate in 20 min	Fructose-6 phosphate, in mg per 1000 mg protein N	Increase in fructose-6-phosphate, in 20 min
Conducting tissues	0	0.11	–	0.42	–
	20	1.18	1.07	4.29	3.87
Petiole parenchyma	0	0.06	–	0.66	–
	20	0.57	0.51	6.65	5.99

Table 3 shows that in 20 minutes there is an accumulation of fructose-6-phosphate, indicating the presence of phosphohexoisomerase in the tissue extracts. On a dry weight basis, the conversion of glucose-6-phosphate into fructose-6-phosphate is twice as rapid in the conducting tissues as in the parenchyma. On a protein nitrogen basis, however, the reverse is true, and the enzyme of the parenchyma is more active. Such a result may perhaps be explained by the fact that the parenchyma always contains much more hexose sugar than the conducting tissues, which typically contain sucrose.

Aldolase

One of the principal glycolytic enzymes is aldolase, which reversibly splits fructose-1, 6-diphosphate into two phosphotriose molecules. There are a number of studies which indicate the wide distribution of aldolase in plants. The enzyme occurs in barley seedlings [30], peas [31], potato tubers [32] and a number of other plants. The highest activity has been observed in young tissues, for example in root meristems [33]. There are, however, no published reports of the presence of aldolase in plant conducting systems. The only attempt to identify this enzyme in conducting tissue was made by Wanner [8] who looked for it in the phloem exudate of Robinia pseudoacacia but failed to find it. It is very probable that this was due to the absence of structural elements of the cell in the phloem sap. Tewfik and Stumpf [33], who fractionated cells of the leaf parenchyma, consistently found that aldolase occurred only in the cytoplasm.

TABLE 4

Aldolase Activity of the Fibrovascular Bundles and Parenchyma of Sugar-Beet Petioles. (in μg of alkali-labile phosphate per gram tissue dry weight)

Expt. No.	Tissue	Time, min			
		30	60	120	180
1	Conducting tissues	125.2	261	437	638
	Parenchyma	110	210	356	422
2	Conducting tissues	224.0	435.7	663.3	842.2
	Parenchyma	75.2	191.4	336.2	453.6

For the determination of aldolase activity we used the method of Herbert and co-workers [34], according to which triose phosphates formed from fructose-1, 6-diphosphate are trapped with KCN or bisulfite. The phosphorus of the triose phosphates was determined after hydrolysis with normal NaOH for 20 minutes at 20°. Enzyme preparations were obtained by extraction of fresh tissues with an equal volume of 0.14 M veronal buffer, pH 7.0, at 4-6° for 25 minutes, after which the sap was pressed through cloth and centrifuged 4 minutes at 3000g. Two ml of sap, corresponding to about 1.2 grams of tissue dry weight, were u ed in a reaction mixture. The reaction mixture contained: 2 ml of enzyme, 2 ml veronal buffer, pH 7.0, containing 0.1 M bisulfite and 0.0125 M fructose-1,6-diphosphate. The total reaction volume was 4 ml. Reactions were allowed ro proceed 30-180 minutes at 37°. They were stopped by the addition of 5% trichloracetic acid.

Results of a determination of aldolase activity are presented in Table 4.

These results show that as in other plant tissues aldolase is present in the conducting tissues and the parenchyma of sugar-beet petioles. It is 1.5-2 times more active in the conducting tissues than in the parenchyma.

Since extracts of fresh tissues were used, it was possible to compare our data with those of James [30], who measured the rate at which fructose-1, 6-diphosphate was split by barley seedling sap.

In his experiments on the effect of 1 ml of sap in 24 hours at 30°, 40 μg of alkali-labile phosphorus was formed. In our experiments with conducting tissues, 640-840 μg of alkali-labile phosphorus was formed in 180 minutes at 37° per gram tissue dry weight; with parenchyma, 430-450 μg was formed.

These comparisons show that the activity of the conducting tissues and the parenchyma is higher than that of barley seedling sap. This indicates an extremely active glycolysis in the tissues we investigated.

Thus, a number of glycolytic enzymes have been demonstrated in the conducting tissues of sugar beet: hexokinase, phosphohexoisomerase, aldolase; phosphoglucomutase was not found. These data show that sugars are degraded to a large extent by the glycolytic pathway in these tissues. Further evidence of this is found in the composition of the phosphorylated compounds of the fibrovascular bundles; hexosephosphates, which are characteristic of glycolysis, are encountered, but not phosphate esters typical of the oxidative pathway of degradation.

The experiments have shown that hexokinase and aldolase are more active in the conducting tissues than in the parenchyma, indicating a more active glycolysis in the conducting system. The heightened glycolytic activity in the fibrovascular bundles is evidently related to the increased intensity of respiration of these tissues.

It is well known that glycolysis is usually the main path of hexose degradation in young, rapidly growing tissues, the meristem of root tips, for example. With respect to the type of metabolism, conducting tissues are similar to meristem in that they possess a heightened glycolytic activity. This is probably due primarily to the activity of the cambium and of the most active phloem cells.

In his study of the phloem sap of Robinia pseudoacacia, Wanner came to the conclusion that in the contents of the functioning sieve cells one could find only the rudiments of a glycolytic system as represented by phosphatase and phosphoglucomutase. On the basis of this he concluded that conducting cells cannot play an active role in the transport of assimilates.

Our results, which indicate an intensive respiration in the fibrovascular bundles [1, 2, 3], also an active glycolysis, are more readily interpreted as evidence for the metabolic nature of translocation in plants.

SUMMARY

Glycolytic enzymes such as hexokinase, phosphohexoisomerase, aldolase and also apyrase, have been detected in the fibrovascular bundles of the sugar-beet plant. No phosphoglucomutase was found. This seems to indicate that the glycolytic process plays an important role in the transformation of sugars in the conducting system of the sugar-beet plant. A confirmation of this is the fact that glucose-6-phosphate and fructose-6-phosphate were always present in the conducting tissues whereas phosphate esters which are typical of oxidative degradation of hexoses were not found in them.

The activity of hexokinase and aldolase was found to be higher in the fibrovascular bundles than in the surrounding parenchyma of the petiole, which means that glycolysis is more active in the conducting system.

Hexokinase could be detected in the conducting bundles throughout the growth period. The highest activity was observed during the period of intense flow of sucrose from the leaves to the root (August). The hexokinase activity in the petiole parenchyma was much weaker and remained almost constant throughout the vegetative period.

The result of the action of hexokinase and phosphohexoisomerase of the fibrovascular bundles and petiole parenchyma is the formation of glucose-6-phosphate and fructose-6-phosphate (in the ratio 85: 15).

The activity of the glycolytic enzymes and the intense respiration [1,2,3] in the fibrovascular bundles compels one to regard the conducting tissues as systems, which are highly active metabolically. It is conceivable that this circumstance is connected with the translocation of substances which takes place in these tissues.

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DISTRIBUTION OF S^{35} -LABELED VITAMIN B_1 IN VARIOUS PARTS OF THE TOMATO PLANT

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In addition to study of vitamin biosynthesis by plants, there is at present a large amount of study of the synthetic capacities of individual plant parts and the provision of these parts with a given vitamin [1-6]. It was shown, for example, that leaves possess the greatest capacity for vitamin synthesis, whereas roots synthesize vitamins either in insignificant quantities or not at all. It is not fortuitous that roots fail to grow on an artificial nutrient medium lacking a vitamin that they do not synthesize [7, 8]. Under natural conditions, however, roots are furnished with vitamins not only by endogenous synthesis but also by movement of vitamins from aerial plant parts and from the soil [9, 10]. It has been suggested that the vitamins B_1, B_6, G, PP, biotin, and others are transported in the plant in a manner analogous to that by which other organic materials are transported. These conclusions were not based on studies using isotopes but on the accumulation of vitamins in the upper and lower portions of the cortex of girdled stems. Such experiments do not afford an opportunity to study in detail the distribution of vitamins in various parts of a growing plant. In addition, there are no data on vitamin levels in various plant parts at a given time, which makes it difficult to assess the physiological role of vitamins in the life of a given plant part and the interaction of the various organs in the plant as a whole. It seemed to us that a study of vitamin distribution might help to explain in even greater detail the promoting effect of externally applied vitamins on plant growth and development [10-13].

We performed the following experiments in order to determine the distribution of vitamin B_1 in the tomato plant, and also to determine how the distribution pattern is affected by the growth stimulator 2,4-D, which is introduced into the fruits.

Pot tomatoes, variety Ground Gribov, which were just beginning to bear fruit, were used. A 0.01% aqueous solution of S^{35} labeled thiamine was introduced into the plants. Selection of this concentration was based on its growth-promoting effect when the solution was applied by sprinkling. Ten ml of solution was applied to each plant through the petiole of a middle leaf. Radioactivity of this amount of solution was 50 μ C. Before application of the solution, the leaflets were removed under water from the leaf in question, after which the petiole was immersed in the solution in a graduated test tube.

The experiment comprised two parts: 1) a study of the distribution of vitamin B_1 in various parts and organs of a normally developing plant; 2) a study of the distribution of vitamin B_1 in plants whose reproductive organs were treated with a stimulating dose of 2,4-D. Four plants were used in each treatment. At the beginning of the experiment the condition of the reproductive organs was assessed using an arbitrary system. Buds, flowers, five-to seven-day ovaries and "dormant" fruits were treated with the growth stimulator. The 2,4-D solution was used in a concentration of 0.001%. In each flower cluster, fruits in approximately the same physiological condition were selected, of which half were treated with 2,4-D (wetted) and half were used as controls. Treatment with 2,4-D was made at the time of application of thiamine. The experiment was begun on May 9 and dismantled on May 14-15, i.e., at the time of completion of thiamine uptake by the plant.

On May 14-15, various organs and parts of the plants were removed, placed in, then weighed and finally fixed at 100-105°, in an oven. The plant material was dried to constant weight at this temperature. The radioactivity of the dried material was determined with a Geiger-Müller counter. The dry material was placed

in a small mortar, moistened with a small quantity of water (to prevent dispersion) and finely ground. An amount of distilled water was then added so as to provide a suspension containing not more than 5 mg dry material in 0.1 ml (to prevent self-absorption). The suspension (0.1 ml) was evenly spread on special brass disks (previously weighed). The disks were then placed on an electric plate at 100-105° to dry the films, and weighed again in order to accurately determine the weight of the dry material. The radioactivity was then determined. With corrections for S^{35} decay, counter efficiency, and the proportion of radioactive to nonradioactive sulfur in the thiamine used, the content of thiamine in μg per g dry weight of each plant part was calculated. Calculation was made in accordance with the relation between the radioactivity of S^{35} and the weight of sulfur atoms corresponding to this radioactivity. Radioactivity was determined in the following parts of the plants: growing points, upper, middle and lower leaves, the nodes and internodes associated with them, the main and lateral roots and the fruits.

TABLE 1

Distribution of Vitamin B_1 in Various Parts of Tomato, Variety Ground (in μg per g dry wt of the organ)

Plant part		Plants not treated with 2,4-D	Plants treated with 2,4-D (0.001%)
Growing points		29.05	25.62
Leaves	Upper	49.05	44.21
	Middle	6.86	0.56
	Lower	0.2	0.03
Nodes	Upper	58.34	63.53
	Middle	48.98	16.31
	Lower	0.99	0.91
Internodes	Upper	49.77	66.72
	Middle	46.58	29.79
	Lower	2.47	0.73
Roots	Main	1.25	0.78
	Lateral	1.35	0.76

Data on the distribution of vitamin B_1 in various parts of the tomato plant, both with and without 2,4-D treatment, are presented in Table 1.

Table 1 shows that in untreated plants the greatest amount of thiamine is accumulated in the upper portions; the greatest concentration occurs in the nodes, with the internodes and the leaves following in that order. It is a striking fact that in the lower aging leaves and other lower parts of the plant the vitamin content is sharply reduced. There is an extremely low level of vitamin in the roots.

These data are strikingly confirmed by the radioautographs (Fig. 1).

As the radioautograph shows, the vitamin is concentrated, for the most part, in the upper growing leaves and their internodes. The high concentration in the leaf veins and the leaflet tips is marked (Fig. 2). A large amount of vitamin is accumulated in the seeds and epidermis of the fruits (Fig. 3).

In the case of treatment of the flower clusters with 2,4-D, the distribution pattern is somewhat altered. There is a decrease in the amount of vitamin in the growing points, leaves and roots, while there is an increase in the nodes and internodes. The vitamin content of the upper nodes and internodes of treated plants is relatively greater, while in the intermediate and lower nodes and internodes it is sharply reduced; there is relatively more vitamin B_1 in the upper and middle internodes than in the corresponding nodes when the plant is treated with 2,4-D.

In contrast with the pattern of distribution of growth stimulators in plants [14], it is interesting that vitamin B_1 is accumulated in the growing points to a lesser extent than in the upper leaves and upper and middle nodes and internodes. Treatment with 2,4-D of the flower clusters causes a reduction of vitamin accumulation in the growing points.

The situation is similar with respect to fruits. While growth stimulators are accumulated in significant quantities in the flowers and ovaries, thiamine accumulation by the fruits is markedly below that of the leaves, nodes, internodes and growing points (Table 2).

The distribution of vitamin B_1 in fruits at different stages is of interest. Reproductive organs — buds and pollinated flowers - in which growth is especially intense, are richer in vitamin B_1, while in unpollinated flowers and "dormant" fruits, in which synthetic processes are less rapid, the thiamine content is considerably lower.

Treatment of the reproductive organs with 2,4-D somewhat changes the distribution pattern. In treated buds, five-to seven-day ovaries and "dormant" fruits, thiamine is accumulated less rapidly, while in unpollinated flowers it is accumulated more rapidly.

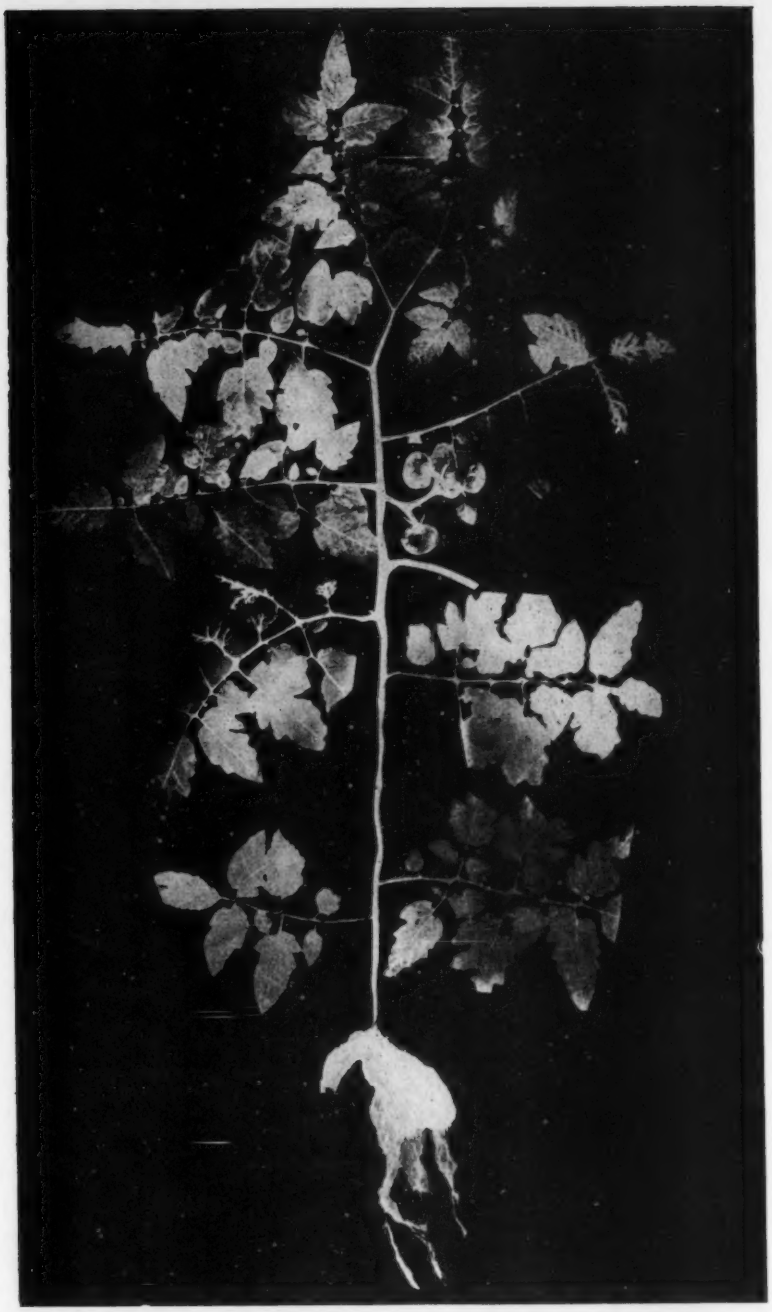

Fig. 1. Radioautograph of a tomato plant — distribution of vitamin B_1 in various organs.

A determination of dry weight made at the end of the experiment showed that with treatment of buds and unpollinated flowers, the accumulation of dry matter was somewhat lower than when 2,4-D had not been applied. For example, in treated buds the dry matter content was 13.25%, while in the controls it was 14.47%; similarly, in unpollinated flowers it was 17.26 and 17.70%. With respect to the total dry matter content of a reproductive

structure the following pattern is obtained. The dry weight of treated buds stays below that of control buds. For example, at the conclusion of an experiment the dry weight of a treated bud might be 6.6 mg as compared with 7.4 mg for a control bud.

It should be added here that no visible growth response of the buds to 2,4-D was noted.

As is well known [15], flowers after anthesis show a marked response to treatment with growth stimulators. A similar behavior was shown in our experiments; at the conclusion of an experiment the weight of treated flowers had increased markedly, being 8.7 mg for a single flower, while that of a control flower was 7.5 mg.

Fig. 2. Radioautograph of the upper portion of a tomato plant.

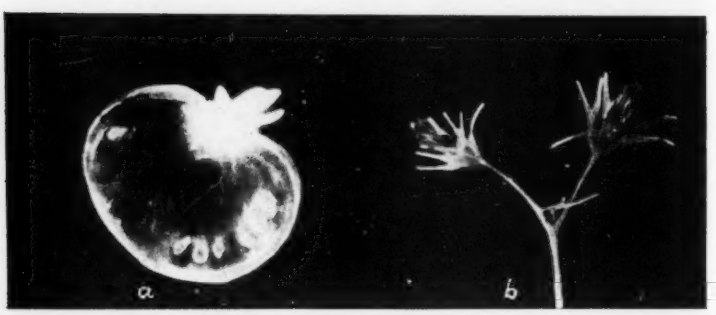

Fig. 3. Radioautograph of a longitudinal section of a tomato fruit (a) and of flowers (b).

On the one hand, therefore, the percentage dry weight of flowers treated with a growth stimulator is lower than that of the controls, and on the other, the dry weight of seven-day ovaries (based on one ovary) which have developed from the flowers treated with 2,4-D increases considerably. This should be regarded as being due to an intensified flow of nutrients to the ovary, which actively responds to treatment. In support of this hypothesis, one may point ot the data of Table 2 showing an increased flow of vitamin B_1 to the ovaries developing from flowers treated with a growth stimulator. At the same time, as Table 2 shows, in reproductive structures which do not respond to treatment, the accumulation of vitamin B_1 not only does not increase but even decreases.

TABLE 2

The Effect of Treatment of the Reproductive Organs of Tomato with 2,4-D on the Distribution of Vitamin B_1 (in μg per g dry wt of the organ)

Condition of reproductive organs at time of treatment	Control	Treatment with 2,4-D
Buds	13.05	9.31
Flowers	8.90	14.53
Five-to seven-day ovaries	14.61	11.96
"Dormant" fruits	4.09	2.68

SUMMARY

Vitamin B_1 introduced through the petiole is distributed nonuniformly in the plant. In tomato plants, vitamin B_1 is distributed as follows: most of the thiamine accumulates in the upper parts of the plant, especially in the nodes, to a lesser extent in the internodes and to even a smaller extent in the leaves. The vitamin is very scarce in the aging lower leaves, in the corresponding nodes and internodes and also in the roots.

Treatment of the reproductive organs with growth regulators exerts a pronounced effect on the rate of accumulation of vitamin B_1. In buds and ovaries not treated with 2,4-D in which growth processes are especially pronounced, thiamine accumulation is noticeably higher, whereas in unfertilized flowers and "dormant" fruits in which synthetic processes occur at a slow rate, the vitamin B_1 content is lower. When the growth stimulator is applied to a reproductive structure, the vitamin response of the latter depends to a great extent on its response to such treatment; thus, accumulation of vitamin B_1 is lower in buds of 5- to 7-day and "dormant" fruits, which do not exhibit a pronounced growth reaction; on the other hand the thiamine content is appreciably larger in flowers, in which the flow of nutrients is enhanced by application of stimulating doses of 2,4-D preparations.

The growth stimulator and vitamin B_1 are distributed differently in the tomato plant. The growth stimulator mainly accumulates in growing points, flowers and ovaries, whereas vitamin B_1 is found mainly in the upper leaves and corresponding nodes and internodes.

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STERILE CULTURE OF THE PLACENTA OF POPPY AS A MEANS FOR THE STUDY OF THE FORMATION OF SEEDS AND THEIR SYNTHESIS OF RESERVE PRODUCTS

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The role of the placenta in the exchange of substances of the plant has been little studied. It has been shown, using the fruit of the opium poppy (Papaver somniferum, L.), that its separate tissues (wall, placenta, seed) possess a specificity in relation to the gas regime, the work of the fermenting systems and the synthesis of several compounds. The placenta in this connection occupies a special position [1, 2, 3]. Based on data obtained on studying the transformation of carbohydrates in ontogenesis of fruits of the poppy and also on the ability of cells of the placenta to accumulate acetic [3] and α-ketoglutaric acid*, one can reach the conclusion that these acids are a result of the decomposition of carbohydrates entering into the placenta from the assimulating organs. The accumulation of acetic acid in the placenta coincides with the time of the most intensive period in the synthesis of oil in the seeds. Thus we hypothesize that the place of deep conversion of carbohydrates (glycolysis) is in the cells of the placenta, and it can be assumed that the products of their decomposition play an important role in the synthesis of reserve products in the seeds. However, we made these conclusions on the basis of indirect data. We cannot obtain direct evidence confirming the indicated hypothesis by studying the conversion of the previously mentioned compounds in the tissues of the fruit not separated from the plant.

In searching for some methods for the determination of the role of the placenta in the metabolism of substances of the fruits, we decided to investigate the method of culturing isolated tissues on a nutrient medium under sterile conditions. The ability of the placenta of poppy to develop independently in proportion to the carbohydrates, and, generally, its ability to maintain and nourish seeds under this condition, would make it possible to use the method of sterile culture for the study of conditions and mechanism of the synthesis of fats and other reserve substances in poppy seeds. LaRue [4] notes the possibility of culturing isolated tissues of the reproductive organs of plants on artificial nutrient media. Based on the above, an attempt was made to culture the placenta of poppy as the most desirable object for an artificial nutrient medium for this goal. We used in our work the methods employed by many other investigators for culturing different plant tissues [5-9], immature embryos, endosperm, the ovaries of fruits [10-13], etc.

METHOD

Fruits of poppy were cut from the plants and sterilized with hydrogen peroxide [9]. Then the placenta was excised from the fruits, using all correct asepsis, if possible with the placenta's vascular-fibrous fascicle. The separate lobes of the placenta along with the seed buds were placed in a test tube or in Koch jars with a congealed nutrient medium. We confined ourselves at the beginning to a Gautheret medium [5] (see table) with glucose (2%) and agar-agar (0.9%). The extract from beer yeast and heteroauxin [5] were among the additional nutrient factors. Not less than 20-25 test tubes with the placenta culture grown in the dark at 25-26° were included in each variant of the experiment. The placentas were cultured from fruits of various ages: one, two, five, six and seven days old [1]. We determined the age of the fruits conditionally from the day of flowering

*Not published

of the plants. One-day-old fruits still maintained the petals of the corolla. Seed buds from one-and two-day-old fruits were still very small and transparent, while those from six-and seven-day-old fruits were not transparent and were more developed. Seeds of seven-day-old fruits already contained starch and fat [14]*. The age of the fruit and seed has great importance for the culturing of the placenta, as we will show further.

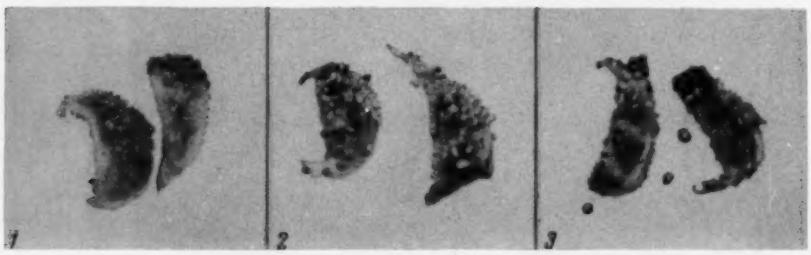

Fig. 1. The placenta of poppy after culturing on a nutrient medium for 23 days. 1) from two-day-old fruits; 2) from three-day-old fruit; 3) from four-day-old fruit. In 1, one mature seed is seen, and the remainder are underdeveloped. In the other cases (2, 3) a significant number of normally ripened seeds survived.

EXPERIMENTAL PART

The placenta placed on the congealed nutrient medium quickly begins to increase in size. Its tissues in the place adjoining the main vascular-fibrous fascicle begin to swell. The placenta from one-and two-day-old fruits grow quite fast in comparison to the placenta from six-and seven-day-old fruits. However only part of the seed buds survive when culturing the placenta in similar conditions. The younger the placenta, the fewer the number of seed buds on it that survive. For example, all seed buds on the placenta from one-day-old fruits perish; in rare instances one survives; two to three seeds survive from two-day-old placentas, at the same time that up to 300 seeds are maturing on every lobe of the placenta under normal conditions. In several cases a significant number of seeds survives (Fig. 1) for five-and six-day-old placentas. All of the seeds that survive on the placenta increased in size, filled out, gradually changed in color, as with seeds formed on undisturbed fruits, and ripened. The process of ripening under conditions of an artificial culture took 23 to 25 days. Ripened seeds separated from the placenta, fell on the medium and sprouted. Sprouts growing on the medium on which the placenta was growing are shown in Fig. 2. It is interesting to note that a sprout from the only seed that survived on one-day-old placenta is seen in the first part of the picture. A picture of shoots after the culturing of five-and seven-day-old placentas is given in Fig. 3. In order to determine if seed buds and seeds could be grown on a nutrient medium without the placenta, the latter were taken from the placenta and placed on the sloping surface of a nutrient medium of the same composition.

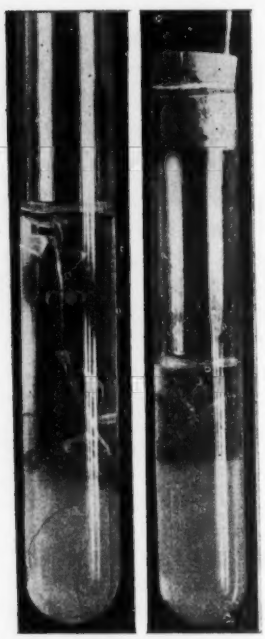

Fig. 2. Seeds from five-day-old (left) and one-day-old (right) placenta, ripening on the culturing of the placenta and the resulting shoots.

Seeds were taken from five-, eleven-and fifteen-day-old fruits for this purpose. Seeds from the five-day-old fruits turned black and died three to four days after planting. Those remaining swelled somewhat, but remained as failures and were underdeveloped. We reached the conclusion from the results obtained that for the given conditions of culturing, the development and ripening of seeds can proceed only on the placenta.

Further work was directed at an explanation of the conditions favorable for the survival of seeds on the placenta after the preliminary

*In the present article, for brevity, the placenta from one-, two-and five-day-old fruits will be called one-, two-and five-day-old placentas.

results showed that the placenta when isolated from the plant is capable of developing an artificial nutrient medium. We investigated the effect of several of the external conditions (moisture, method of placing the placenta on the agar), sources of carbon and nitrogen, the composition of the mineral environment and additional factors of nutrition.

The effect of moisture. According to the data of Prokof'ev and Kholodova [15], the atmosphere of the cavity of young fruit of poppy, in which the seeds are located, has a relative humidity of 100%. Therefore, it was natural to assume that the conditions of moisture in the test tubes with agar did not meet the demands and

Fig. 3. Sprouts from seeds ripened in a culture on placenta from five-day-old fruit.

the seeds dried. However, attempts to increase the moisture by placing the culture test tubes and Koch jars in a box with a water seal did not increase the success. The condition of increased moisture proved to be unfavorable for the placenta and did not affect the survival of seeds on the placenta.

Poor results were obtained from the immersion of the placenta in agar: immersing even only two-thirds of the placenta in agar results in quick blackening and death. Just the same it seems to us that the survival of seeds on the placenta must depend most of all on the composition of the nutrient medium and, in part, on the presence and nature of additional growth factors. As we know, the additional factors of growth often have a decided importance on the culturing of growing tissues [12]. We took the basic Gautheret medium and varied its composition further beginning with tests of the concentration of heteroauxin and extracts from beer and baking yeasts. The addition of heteroauxin and substances found in the yeast extract was necessary for good growth of the placenta. The placenta was not older than four-days- usually one-and two-days-old - for all the latter experiments. We assumed that the younger the placenta, the less would be the accumulation of assimilates in it which would show a reaction on the development of seeds.

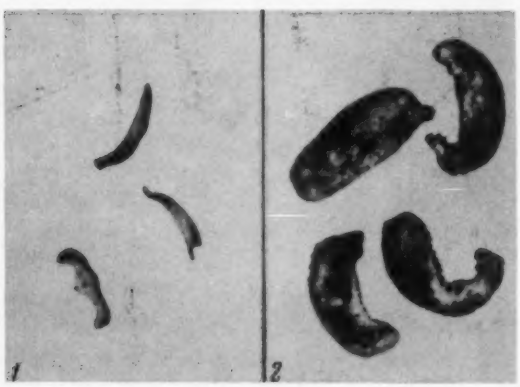

Fig. 4. Placenta up to placing on a nutrient medium (left) and after culturing for eight days on a Gautheret medium with glucose and yeast extract.

Heteroauxin. It was found that the optimal concentrations of heteroauxin (in the presence of yeast extract) are 0.05 to 0.1 ml/l, although the placenta maintains its concentration at 0.5 ml/l.

The placenta at the place of contact with the medium begins to turn black after three days using 1 ml/l, and after a week dies.

Yeast extract. We studied the extracts from beer and baking yeasts prepared according to Gautheret [5]. They were added to the medium in the quantity of 2% together with the heteroauxin. Both of the extracts were

distinguished by their effect on the growth of the placenta and by the survival of seeds on the placenta. Fewer seeds survived on the placenta with the extract from baking yeast, in fact,often they did not survive, and a significantly stronger growth of the placenta itself was observed. Its volume increased several times in the seven days of culturing (Fig. 4). An increase up to 4% in the concentration of the extract from baking yeast brought about even more intensive growth of the placenta. However, fast blackening of the tissue in contact with the surface of the agar and then the rapid death of the placenta showed the harmful effect of the high concentrations of yeast extract.

Source of carbon. The study with the sucrose (2-3%) and glucose (2%) showed that the placenta grew more intensively on the glucose medium than on the sucrose. The placenta held a higher concentration of heteroauxin on the medium with glucose than with the sucrose. For example, the placenta on sucrose was already dead after a week with 1 ml/l, but only a slight yellowing of the tissue appeared in this period with the glucose. The noticeably better growth of the placenta and the high survival of seeds were observed on other mediums(White and Heller), also with glucose. On the basis of the points indicated above, we made the conclusion that glucose is a better source of carbon than sucrose for the placenta of poppy. However, most of the work on the culture of organs and tissues is carried out on sucrose. We think that this inconsistency can be explained by the individual features of the object. Analysis of carbohydrates of the yound placenta showed that the quantity of monosaccharides (fructose and glucose) is significantly greater than the quantity of sucrose.

TABLE

The Composition of Nutrient Media Used for Culturing the Placenta (in mg/l)

Composition of the nutrient medium	Gautheret	White	Heller	Nitsch
1. $Ca(NO_3)_2$ anhydrous	500	200		
2. KNO_3	125	80	600	2000
3. $MgSO_4 \cdot 7H_2O$	125	36.0	250	250
4. KH_2PO_4	125			
5. Na_2SO_4	—	200		
6. KCl	—	65	750	1500
7. $NaH_2PO_4 \cdot 2H_2O$	—	16.5	125	250
8. $FeCl_3$	1.0	—	—	
9. $Fe_2(SO_4)_3$	—	2.5	—	
10. $CaCl_2 \cdot 6H_2O$	—	—	75	25
11. P-indolacetic acid	0.05—0.10	—	0.1	0.1
12. Yeast extract	20 ml		20 ml	20 ml
13. Bertlo mixture *	10 drops		10 drops	10 drops
14. $MnSO_4$	—	4.5		
15. $ZnSO_4$	—	1.5		
16. H_3BO_3	—	1.5		
17. KI	—	0.75		
18. Glycocol	—	3.0		
19. Nicotinic acid	—	0.5		
20. Pyridoxine	—	0.1		
21. Thiamine	—	0.1		

*Following composition (in mg/l):

$Fe_2(SO_4)_3$	50	$CoCl_2$	0.05
$MnSO_4$	2	$ZnSO_4$	0.1
KI	0.5	$CuSO_4$	0.05
$NiCl_2$	0.05	H_3BO_3	0.05

H_2SO_4 at 66° Baume, 1 ml

According to all the data, the assimilates in plants go into the placenta in the form of sucrose, which then undergoes hydrolysis and is further converted to a monosaccharide, largely glucose. The observed increase in the concentration of sucrose in the placenta with the age of the fruit can be explained by the dying out of synthesizing processes in the seeds. We used glucose in the quantity of 20 g/l for nutrient mediums.

Organic nitrogen. Given the fact that the basic synthesis of amino acids in plants takes place in the roots [16,17] one can assume that the placenta possesses limited ability for the synthesis of amino acids and it cannot supply all of the demands of itself and the seeds for amino acids in proportion to the produced mineral nitrogen. Replacing nitrates on the Gautheret medium with asparagine in the quantity of 0.1% resulted in the very rapid growth of the placenta. However the seeds on the placenta had low survival and quite soon at the place of contact between the tissue of the placenta and the medium, blackening began to spread out to the entire placenta and led to its death. The effect of other amides and amino acids was not studied by us.

Bertlo mixture. Bertlo mixture is added to nutrient mediums by many authors. In our experiments with the placenta it did not show its specific effect on the survival of seeds. Nevertheless, this mixture was added to the latter cultures in the quantity of 10 drops to a liter to all mediums with the exception of the White. Ferric chloride was excluded from the Gautheret medium when the Bertlo mixture was added.

Other nutrient media. The composition of the other media that were used is given in Table 1. The White, Heller and Nitsch media did not show any advantage in relation to the Gautheret medium. The placenta grew on all of these mediums with greater or lesser intensity. Meanwhile, the survival of seeds was not greater, and was sometimes even less, than for the Gautheret medium; survival was determined mainly, not by the composition of the mineral salts, but by the heteroauxin, yeast extract or the additions according to White.

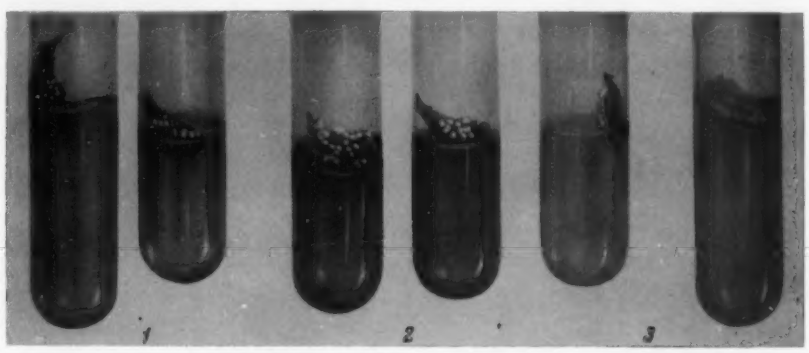

Fig. 5. Placentas from one-day-old fruits. 1) On a Nitsch medium with adenylic acid; 2) on a Gautheret medium with coconut milk; 3) on a Nitsch medium with yeast extract. After 16 days of culturing.

Up to four seeds survived on the one-day-old placenta when heteroauxin and yeast extract with α-naphthyacetic acid (0.1 mg/l) was substituted on the Nitsch medium, but the placenta unexpectedly turned black prior to the beginning of the ripening of the seeds, and soon died.

It was explained that when comparing the character of the growth of the placenta for all forms of the nutrient media their composition shows an effect mainly on the growth of the placenta itself, and is reflected little in the survival of seeds. The indicated media result in either very intensive growth of the placenta with the formation of protuberances and swellings, or in slower growth on which the normal appearance of the placenta is maintained. As we indicated above, the substitution of heteroauxin and yeast extract with α-naphthyacetic acid or the White additions (table) leads to the survival on one-and two-day-old placentas of not more than five seeds. Consequently, we failed to obtain the survival of seeds on the very young placenta using nutrient media and added nutrient factors, while at the same time the growth of the placenta itself was very good.

The conclusion was reached on the basis of the facts obtained that the use of added nutrient factors for nutrition of seeds is insufficient and something further is needed, without which, it seems, the normal development of seeds cannot be carried out. We then studied the action of adenylic acid and coconut milk.

Adenylic acid. Adenylic acid was added to all of the above nutrient mediums in the quantity 10 mg/l (the heteroauxin and yeast extract were not put in the medium). Placentas from one-, two-and three-day-old fruits were used for this culture. The presence of adenylic acid retarded the growth of the placenta. This retardation was observed for all media. The external appearance of the tissue changed at the same time. With adenylic acid the tissue of the placenta maintained a white color and a "dry" consistency during the whole time of the culturing (as in the natural state of the fruit). In other cases, especially when the placenta grew very rapidly and the seeds were absent, the placenta sometimes acquired the appearance of a sponge saturated with water during the day from 12 noon to 3 P. M.

The presence of adenylic acid showed a noticeable effect on the survival of seeds on the placenta. However, its role was not the same on other media. The weakest effect of all was when adenylic acid was used with one-and two-day-old placentas on the White and Heller media. It showed a noticeable effect on the Gautheret medium. The best results were given by the Nitsch medium. The one-day-old placenta retained 15 to 20 seeds on this medium at first. These seeds filled out normally and ripened (Fig. 5, 1). Many of the seeds of the three-day-old placenta survived on the White medium.

Coconut milk.* Coconut milk was added to the Gautheret and Nitsch mediums in the concentrations of 9, 10, and 16%. Coconut milk gave better results in comparison with the adenylic acid. The one-day-old placenta retained up to 30 to 40 seeds (Fig. 5, 2) and sometimes 20 to 30 on the Gautheret medium. The seeds without the placenta from one-and two-day-old fruits placed on the same nutrient solution quickly died; the four-day-old placenta on the medium with coconut milk (10%) retained practically all of the seeds (Fig. 6, 1, 2). The seeds

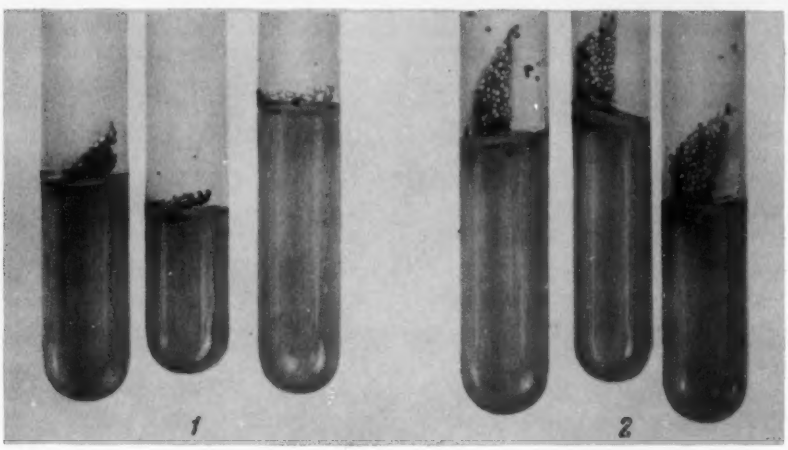

Fig. 6. Placenta after 6 days of culturing on a Gautheret medium with coconut milk. 1) From two-day-old fruit; 2) from four-day-old fruit. In number 1 there are comparitively few seeds; in number 2 the seeds survived completely.

isolated from the four-day-old fruit, in contrast to the younger, increased in size over a fairly short period of time, but soon darkened and remained undeveloped. Thus nearly all seeds survive on the older aged placenta on the medium with coconut milk and are carried through up to total ripening, while seed buds quickly stop their development and remain undeveloped without the placenta. Coconut milk in the quantity of 16% suppresses the growth of the placenta.

It must be stressed when considering the results of the experiment with adenylic acid and coconut milk that their presence in the medium increased the number of seeds surviving on one-and two-day-old placentas 20 to 30 times, and the seeds on the four-day-old placenta practically infinitely. Neither the adenylic acid nor the coconut milk were able to guarantee the development of seeds on the nutrient medium without the placenta.

*Coconut milk from two lots was used: from Africa, from the French Institute of Black Africa, and from China from the island of Hainan, sent by Professor Je-K'ann.

However Maheshwari [18] recently showed the possibility of the development of seed buds of poppy on a congealed nutrient medium up to their complete ripening. Maheshwari used a Gautheret medium with heteroauxin and kinetin [19]. The substance determining the development of the seed buds in the given case was kinetin.

Kinetin. We investigated the action of kinetin on the growth of the placenta and on the development of seeds on the medium without the placenta in connection with the work of Maheshwari. We used a Gautheret medium (table) with kinetin (0.4 mg/l) and heteroauxin (5 mg/l) for these experiments, as did Maheshwari. In several cases α-naphthyacetic acid (0.1 mg/l) was used in place of the heteroauxin. The kinetin was also studied on the Nitsch medium (0.8 mg/l); the Nitsch medium without kinetin and with α-naphthyacetic acid (0.1 mg/l) was used in a similar manner.

Two- and four-day-old placentas were placed on the Gautheret medium. Both showed good growth, the two-day-old placenta did not retain any seeds and very few seeds survived on the four-day-old placenta. However they ripened normally. The placenta grew somewhat more poorly on the Nitsch medium possibly because of the high concentration of kinetin (0.8 mg/l); with α-naphthyacetic acid more seeds survived on the one-day-old placenta than with kinetin.

The effect of kinetin, and also the effect of coconut milk, on the development of seeds without the placenta is manifested differently in relation to the growth of the seeds. Seeds from two-day-old fruits quickly died and the seeds from four-day-old fruits increased in size for some time, but after about a week turned black and remained undeveloped. From the outside they were similar to those on the medium with coconut milk. Therefore kinetin did not show any effect on the development of seeds on the placenta in the conditions of our experiments. It also did not show a decided effect on the development of seeds separated from the placenta.

The age of seeds. We pointed out in the beginning of the article that the survival of seeds on the cultured placenta depended on their age. Usually one seed on one-and-two-day-old placentas, a maximum of three to four (on the two-day-old), survived on media with yeast extract and heteroauxin. Up to 40 to 50 seeds survived on the five-day old placenta in these same conditions (Fig. 1). 20 to 30, and up to 40 seeds, survived on the two-day-old placenta with coconut milk on the Gautheret medium, and nearly all survived on the four-day-old placenta (Fig. 6, 1, 2). Thus, the younger the placenta, the more demanding it is for additional growth factors; the older the placenta, the easier it is to culture. There are in the literature [12] similar indications of the role of the age of embryos in relation to their culturing on artificial nutrient media. We found in our own experiments with the placenta of poppy that some substances, unknown at the present time, which are concentrated in the endosperm of immature coconuts make possible the cultivation of very young placentas with the survival on them of 10 to 15% of the seeds and on the older placentas with the survival of practically 100% of the seeds.

Returning to the question of the role of kinetin, we must note that Maheshwari cultivated seed buds of a specific age, after fertilization and division of the egg cell (two-celled proembryo) [18]. It is possible that the four-day-old seeds in our experiments were younger, in connection with which we obtained other results*. It is thought that the seed buds in one-and two-day-old fruits of poppy were still not fertilized. We suggest that the question of the age of seed buds and the stage of their development is very essential for understanding the character of their development in an artificial culture. Therefore, carrying out further cytological investigations of seeds cultivated on the placenta and without the placenta would be of undoubted interest.

SUMMARY

1. The investigations carried out showed that the placenta of poppy (Papaver somniferum L.) can be cultivated when isolated from the plant on a congealed nutrient medium in sterile conditions. The intensive growth of the placenta was observed on nutrient mediums of various compositions (Gautheret, Nitsch, Heller, White) with glucose, yeast extract and heteroauxin.

2. The character of the growth of the placenta in conditions of isolated culture depends on its age. The very young placenta from one-day-old fruit grows without seed survival; from two to five seeds survive from two- and three-day-old fruit. Up to 40 seeds survive on the placenta from five-day-old fruit. The seeds remaining on the placenta develop normally, ripen and exhibit the ability to sprout.

*It must be noted that during the vegetation period in 1958, the average temperature was lower than the normal average temperature from June to August. This led to the slow development of fruits and seeds on the experimental section of the institute.

3. The use of adenylic acid and especially coconut milk sharply increased the quantity of seeds surviving on the cultivated placenta. From 15 to 40 seeds survive on the placenta from one-day-old fruit and they ripen; practically all seeds survive and ripen from the four-day-old.

Kinetin does not show an effect on the survival of seeds on the placenta from one-, two- and even four-day-old fruit. Seeds of this same age, when isolated from the placenta, are not able to develop on the nutrient mixture of the same composition. Four-day-old seeds swell under the effect of coconut milk and kinetin, but remain undeveloped.

4. The developed method of sterile culture of the placenta of poppy can be used for the study of the formation of the seeds and the mechanism of their synthesis of fat and other reserve substances.

M. Yasnitskaya, a student in the fourth course at Gor'kii State University, took part in this work.

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STIMULATION OF PLANT GROWTH BY X-RAY IRRADIATION

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There are many data in the literature on the stimulation of plant growth by ionized light [1]. In recent years this question has been discussed in a series of conferences, including the International Conference on Peaceful Use of Atomic Energy in Geneva [2-8]. However, up to the present the findings have not been clear. This can be explained by the intricacy of questions which deal primarily with morphological investigations and also by the fact that the phenomenon of growth itself has not been determined exactly and every investigator has his own ideas.

We will consider the effect of x-ray irradiation on growth in broader terms as the increase of the linear dimensions and dry mass of the plant. Such terms do not reflect the actual phenomenon of growth as it was formed, for example, by Bauer, but it can be so considered because the linear dimensions and accumulation of dry mass largely characterize the stimulating action of ionized light.

EXPERIMENTAL DATA

Experiment 1. The object of the experiment was to compare the intensity of growth of plants of winter wheat 599 irradiated when four days old with doses that could hypothetically be considered stimulating. Plants were grown in Koch jars, as shown in the figures (with four repetitions) on an A. Mayer nutrient solution in a luminostat at constant light and a temperature of 23 ± 2°. Irradiation was carried out with an RUM-3 apparatus without a filter for the following conditions: 15 ma, 180 kv, focus length 17 cm, intensity of the dose 530 rem. Doses of irradiation: 25, 50, 100, 500 and 1,000 r. All plants were cut off at the level of the coleoptile immediately after irradiation and following this, they sprouted from this level. Data on the dimensions of the leaves of all series six days after irradiation are given in the table. Plants that were irradiated with doses of 500 and 1,000 r are shown in Fig 1 as they appeared seven days after irradiation.

As we see, there is no noticeable difference in the sprouting of severed leaves of plants not irradiated and those irradiated with doses of 25, 50 and 100 r. No difference appeared in the following month and a half during which observations were made. A dose of 500 r had a clearly delaying effect on growth activities (on the leaves of the second stage) and there was a still more strongly expressed effect for a dose of 1000 r.

We will give some attention to the retardation of growth of a dose of irradiation and follow how the linear dimensions of the plants change though time. Plants that were not irradiated and those irradiated with doses of 500 and 1,000 r are shown in Fig. 1, eighteen days after irradiation. The plants were successively cut immediately after irradiation, three days after irradiation and five days after irradiation; the basic form of the vegetative mass is leaves of the third stage.

The irradiated plants of both series sprouted significantly more heavily than the nonirradiated plants up to the time of photographing, and, in addition to this they had longer leaves. In comparison with what was given earlier, the position has consequently changed. Such a result is not accidental. We found this in all experiments that used noticeably high doses of irradiation. However, in the experiments of longer duration, this additional picture is cleared up.

Experiment 2. Sprouts of the same winter wheat 599 were irradiated with a dose of 1000 r under the conditions described above, and following this the nonirradiated and irradiated plants were grown in the luminostat

Fig. 1. Sprouting plants of winter wheat 599, irradiated when four days old and severed at the level of the coleoptile immediately after irradiation. 1) Not irradiated; 2) irradiated with dose of 500 r; 3) irradiated with a dose of 1,000 r. Photo taken seven days after irradiation.

first in Koch jars and then in glass containers on a nutrient solution. Plants 22 days after irradiation are shown in Fig. 3.

Up to the time of photographing, the nonirradiated plants had four leaves, the irradiated plants five leaves; that is, the irradiated plants developed faster. In addition to this they were also taller. This repeats, then, the findings shown for Experiment 1.

Fig. 2. Relative height of plants of winter wheat 599 irradiated when four days old and successively cut after irradiation, on the third day and on the fifth day after irradiation. 1) Not irradiated; 2) irradiated with doses of 500 r; 3) irradiated with dose of 1,000 r. Photo taken 18 days after irradiation.

Otherwise they all appear to be the same plants 36 days after irradiation (Fig. 4). Up to this time, the difference in tempo of development of the nonirradiated and irradiated plants is to a significant degree obliterated and up to the time of photographing all had a similar number of leaves, six, although the development of the sixth leaf for irradiated plants proceeded somewhat faster, and was also longer.

TABLE

The Effect of X-ray Irradiation on Sprouting of Leaves (length of the sprouting portion of the leaves, cm)

Leaves	Not irradiated	Doses of irradiation				
		25 r	50 r	100 r	500 r	1,000 r
First stage	9.3±0.3	9.3±0.3	9.0±0.3	9.0±0.3	9.4±0.3	8.8±0.2
Second stage	20.0±0.30	19.7±0.4	20.8±0.4	19.3±0.4	16.1±0.3	8.6±0.2

Thus, the stimulating action of the x-ray irradiation on the linear dimensions of the leaves of plants of winter wheat 599 appeared only in a specific period of development and then disappeared. This period is connected with the development of leaves during the time of irradiation in an embryonic condition in the leaf sheath. Leaves developing at a later date are not affected by this stimulating action, and they exhibit only the oppression that was observed for plants in the first week after their irradiation.

In connection with the stimulating action of x-ray irradiation on the linear development of leaves, the following must be noted also. Lengthening of leaves of irradiated plants invariably is accompanied by decrease in the width of the leaf blade. This is seen graphically in Fig. 3.

Finally, one more fact must be noted. If the irradiated plants in the indicated periods of time surpass the nonirradiated plants in leaf lengths, then the leaves themselves, after their growth was completed, were smaller for irradiated plants. This is well seen from the comparison of the length of the leaves of the different stages for nonirradiated and irradiated plants in Fig. 4.

Experiment 3. Four-day-old shoots of winter wheat 599 were irradiated with doses of 500 and 1,000 r in the same conditions as before, and immediately after irradiation were severed at the level of the coleoptiles. The growth of the plants proceeded in a luminostat on a nutrient solution in Koch jars (see Fig. 1 and 2). Five days later the growing parts of the leaves were again cut back to the level of the coleoptiles, weighed and dried to a constant weight. This operation was repeated five days later. Changes in the weight of leaves under the influence of the x-ray irradiation showed the following (weight of the leaves of 90 plants in g):

1) Five days after irradiation:

		% of dry substance	Difference
Not irradiated	0.3640±0.0198	9.60±0.23	
500 r	0.3700±0.0107	10.30±0.23	0.70±0.33
1000 r	0.2930±0.0015	11.30±0.23	1.70±0.33

2) Ten days after irradiation

		% of dry substance	Difference
Not irradiated	0.2494±0.0339	9.50±0.00	
500 r	0.2906±0.0230	1,025±0.05	0.75±0.05
1000 r	0.2396±0.0094	12.40±0.10	2.90±0.10

The indicated data show that the dry weight of sprouting leaves does not change under conditions of irradiation with a dose of 500 r. There does not appear to be a mathematical difference in relation to leaves of nonirradiated plants. However, there is the tendency toward a slight increase, especially ten days after irradiation. The dry weight of leaves five days after irradiation is decreased under the influence of irradiation with a dose of 1000 r, and the mathematical difference is apparent. But ten days after irradiation the dry weight of the leaves of irradiated plants was equal to that of the nonirradiated plants to the extent that the difference was in the range of the experimental error.

It is important to note the following. As we saw on irradiating with a dose of 500 r, the increase in the linear dimensions of the leaves is clearly suppressed in the first week after irradiation (see table and Fig. 1). Meanwhile the dry weight of the leaves at this time does not decrease, but even has a tendency to increase.

Even greater is the tendency to increase ten days after irradiation, when the linear dimensions of the plants also begin to increase. The reason for this is the increase in the percent of dry substance in the leaves.

DISCUSSION OF RESULTS

The indicated experiments showed that when shoots of winter wheat 599 were irradiated with doses of 25, 50 and 100 r, there was no observable stimulating or depressing effect on the linear dimensions of plants. Irridiation with doses of 500 and 1000 r resulted in a decrease in the linear dimensions of leaves. However, the position is changed after this. Two to three weeks after irradiation, plants which showed a depressed growth during the first week outgrew nonirradiated plants, and based on the same justification could be considered as stimulated. But even later the position changes again and irradiated plants stand out from nonirradiated plants in their linear dimensions. Thus, there appears to be a superficial effect of depressing and stimulating growth of plants when judged by the changes in linear dimensions of the leaves.

Fig. 3. Relative size of plants of winter wheat 599 of nonirradiated plants (left) and plants irradiated with a dose of 1,000 r while in the sprouting condition (right). Photo taken 22 days after irradiation.

Actually in this case there is no stimulation of growth. There is only a stimulation of leaf formation in a specific stage of the plant's development [10]. Under the influence of irradiation, several of the leaves which appear begin to develop faster, and the impression of more intensive growth has been given during this specific period. Then this effect disappeared. All leaves of irradiated plants that had completed their growth, as we saw, soon corresponded to the leaves of nonirradiated plants.

This seemingly more intensive growth in the described experiments where one to four-day-old shoots were irradiated appeared on leaves of the third, fourth and fifth stages, which in the period of irradiation still had not emerged from the sheath and were, to a known degree, in the embryonic form. The idea of the "stimulating" action of x-ray irradiation with doses of 500 and 1000 r was based on these.

The difficulties in understanding the facts of the increase of dry weight of leaves of irradiated plants are not described. As was shown earlier, the processes of mobilization of plastic substances from the seed, the photosynthesis of the substances and their transformation are not stopped when the shoots and seeds are under the influence of irradiation, even when very large doses are used, but are only limited to a greater or lesser degree, depending on the dose. In connection with this, conditions are created in which the supply of plastic substances to the cells is converted for use in growth when irradiating with doses that do not completely suppress growth, and these substances accumulate. For this the dry weight of the cells and the entire organs increases. However, this by no means indicates growth insofar as the plastic substances are not transformed into protoplasm. Thus, there are also data in the above described experiments on the relative increase of the dry weight of leaves of irradiated shoots.

Such is the nature of the "stimulating" action on growth of x-ray irradiation in the experiments described above. The disclosure of this characteristic makes the presentation of facts on the increase in linear dimensions of irradiated plants up to the present time understandable. At the same time, this characteristic does not encompass all cases of the effect of stimulation connected, for example, with breaking the dormancy of seeds and buds, increasing the formation of reproductive organs and earlier flowering and maturing of plants, etc. All cases of this type will be looked at later using concrete data of observations and experimental investigations.

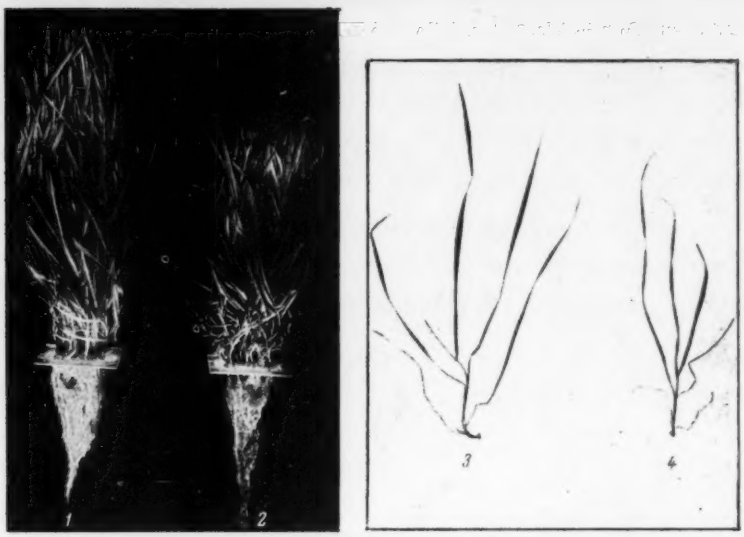

Fig. 4. Same plants as in Fig. 3, 36 days after irradiation. 1 and 2) Plants taken from the containers; 3 and 4) individual plants; 1 and 3) non-irradiated plants; 2 and 4) irradiated plants.

SUMMARY

The linear dimensions of winter wheat (variety 599) seedlings were not affected by X-ray doses of 25, 50 or 100 r. Doses of 500 and 1000 r led to a decrease of the linear dimensions of the plants. However, later on, the situation radically changed. Two or three weeks after irradiation plants in which growth was inhibited during the first week overgrew the nonirradiated plants and in respect to length of leaves could be regarded as having been stimulated. Even much later the situation again changed; the size of leaves of irradiated plants were found to be smaller than those of the nonirradiated plants. The foregoing is a superficial description of inhibition and stimulation of plant growth as judged by the linear dimensions of the leaves.

In reality there is no growth stimulation. What one really observes is stimulation of leaf formation at a definite stage of development of the plant. This occurs when leaves in the rudimentary state are irradiated. After irradiation such leaves become longer but ultimately turn out to be narrower.

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THE CHANGE IN THE RHYTHM OF DEVELOPMENT AND GROWTH OF SUMMER WHEAT UNDER THE INFLUENCE OF SEVERAL YEARS OF CULTIVATION IN THE DESERT

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Among the symptoms and characteristic that guarantee the successful protection of the plant from the harmful effect of drought, attention is given, in general, to biological characteristics of plants, among which the role and importance of the character of the rhythm of development and growth must be stressed.

The adaptation of this or other species of plants to the specific natural conditions of the external environment depends on the ability of the plants to adapt their cycle of development to these conditions. The normal course of the process of development and growth is possible only under conditions matching the rhythm of development and growth with conditions of the surrounding environment. "The individual development is the basic source of genetic changes on which evolution is based" [1]. The effect of the external conditions on the processes of individual development is shown by the rate of the passing of the stages of development [2]. Nonconformity between the conditions of the environment and the natural demands of the plant involves a change in the rate of passing through the stages of development. The plant under the effect of drought either must change its rhythm of development sufficiently to meet these conditions, or die. Data of a number of authors [3-8] bear witness to the significant reaction of wheat to drought expressed in changes in the rhythm of development. Thus, a western Siberia selection slows down in its development under conditions of drought (first biotype, according to Udol'skaya), as the type Povolzh'ya (second biotype, according to Udol'skaya) develops without noticeable delay in these conditions [5,6]. Representatives of the first biotype, in spite of the retardation of development in the period up to the formation of the spike, shorten, to a significant degree, the second half of the vegetation period, but ripen almost at the same time as the control.

The relationship of drought resistance to the rhythm of development generally is shown graphically when plants are examined for drought resistance in various soil and climatic conditions.

Type Mil'turum 321 in the regions of western Siberia is considered highly drought-resistant, but it appears to be slightly resistant upon examination in Povolzh'. On the other hand type Nizhnii Povolzh', which has high drought resistance in its native area, shows weak resistance in the Kuban [6].

The significant changes in the rhythm of development are observed under conditions of wilting. Thus, wilting occuring during the phase of stem development leads to a delay in spike formation, but if it continues into the period of spike formation and flowering, a more rapid ripening is observed [7]. The transfer of 40 down to 20% of the moisture of the soil (based on full capacity) causes a decrease not only of the period of spike formation to ripening, but also of the period from planting to spike formation [8]. The indicated data show that an insufficient water supply has a large effect on the path of development of summer wheat, upon which the size of the harvest is dependent.

The present investigation is devoted to a study of the changes of the rhythm of development and growth of summer wheat in connection with many years of cultivation in the arid conditions of the desert of Betpak-Dala.

The experiment was carried out in field conditions in the Pribalkhash desert (Alma-Ata region) in the valley of the Kulan-Basa. Different reproductions of soft summer wheat Eritrospermum 841 were compared:

Saratovskaya, Dzhezkazganskaya, Alma-Atinskaya, Karagandinskaya, and also type Grekum 1538 (the Dzhezkazganskaya reproduction, harvested in 1948).

Planting of the wheat in all years of the study was done on spring-cultivated wide rows 35 cm apart normally planted at 20 kg/hectare. The plowing depth was 25 cm. Reproductions were planted in alternating rows of squares of 200 to 400 square meters.

The observations of the changes in the rhythm of development were carried out by means of statistical calculation of the number of stems (in the row, 10 m long) living through to spike development and to flowering and also the number of stems with ripened spikes, on every day for the entire period of spike development, flowering and ripening. The spikes were cut off daily at the time of ripening and the number of fully ripe spikes were counted. The stage or ripeness was taken as the time of complete drying of the spike scales. Determination of the dry substances was made by means of drying and weighing 100 plants, and the elements as a component of the structure of the harvest were measured according to the Stankov method [9].

Type Eritrospermum 841 was investigated on the Krasnokutskii selection station in the Rostov region where it grows naturally. The Dzhezkazganskaya reproduction was cultivated for a period of many years (seven) in the severe conditions of the unirrigated areas in the northwest part of the Betpak-Dala desert. The Alma-Atinskaya and Karagandinskaya, on the other hand, were cultivated for a period of time in more moist zones: first, on the unirrigated areas in the foothills of Zailiski Ala-tau, second, on experimental area in the Karaganda region.

TABLE 1

The Size of Leaves of Different Reproductions of Summer Wheat by Stages, in cm (calculated from bottom to top, during the phase of milk ripeness)

Reproduction	1		2		3		Average area of the three stages, cm^2
	Length	Width	Length	Width	Length	Width	
Saratovskaya	12.6	0.74	11.2	0.87	8.4	0.74	75.67
Dzhezkazganskaya	12.6	0.64	12.5	0.77	8.9	0.72	72.42
Karagandinskaya	11.0	0.72	12.0	0.77	10.3	1.03	84.92

Many years of cultivation of a type in specific soil and climatic conditions that are absolutely distinct from the conditions where the characteristics of this wheat were developed must bring about a change in the developed rhythm and growth in the native area of the type, as described for the Saratovskaya (maternal) form. The investigation of all the indicated reproductions in the same conditions would make clear the direction and degree of these changes. We have a basic interest in the description of the Dzhezkazganskaya reproduction, an example of which would be to follow the direction of the change in the rate of development which is one of the essential features of the adaptive reaction to drought.

Variety Grekum 1538 was used in the experiment with the goal of determining whether the differences between the reproductions pass the limits of the variety differences or if they remain within the limits of variety differences.

A short description of the soil and climatic conditions of the place where the experiments were carried out is given in one of our works [10]; therefore we will not dwell in detail on this question but will make only the following observations. The spring-summer period of 1949 was relatively moist for the area of our investigations (170 mm); on the other hand, in 1950 and 1951, the spring was dry and cold and the summer hot with strong winds and often with dust storms accompanied by a lowering of the relative humidity of the air to 10%. There was not one rain that could penetrate the soil more than 5 cm for the entire vegetation period of 1950 and 1951. In connection with this, the rhythm of development of the study plants is different for the different years. In 1949 the Dzhezkazganskaya reproduction ripened on July 3, but in 1950 and 1951 ripening occurred on

TABLE 2

Growth (in cm) and Accumulation of Dry Substance (in g per plant)

Reproduction	Growth				Dry Substance			
	Sprouting	Stem development	Spike development flowering	Milk ripeness	Sprouting	Stem development	Spike development flowering	Milk ripeness
1949	14/V	30/V	17/VI	22/VI	14/V	30/V	17/VI	22/VI
Alma-Atinskaya	9.1	20.4	38.1	43.7	20.3	100.4	168.3	176.2
Dzhezkazganskaya	10.2	20.2	37.4	42.5	22.6	98.7	163.0	167.8
Grekum 1538	8.3	19.0	34.5	40.2	18.0	94.2	158.7	161.7
1950	14/V	31/VI	20/VI	28/VI	15/V	12/VI	18/VI	27/VI
Saratovskaya	12.4	27.3	35.6	36.4	9.7	91.20	140.7	144.6
Dzhezkazganskaya	12.6	23.6	31.1	32.1	9.8	85.6	132.2	134.4
1951	5/VI	15/VI	27/VI	6/VII	2/V	19/V	27/VI	8/VII
Saratovskaya	7.1	28.7	36.9	37.8	10.4	19.1	57.1	91.0
Dzhezkazganskaya	8.3	24.5	32.7	33.2	11.2	18.9	54.7	83.4
Karagandinskaya	10.1	29.6	35.3	37.9	12.2	20.4	61.2	105.6

July 13 and 11, respectively. However, if we consider that planting was carried out 20 and 24 days earlier in 1949 than in 1950 and 1951, then, as a result, we see that the plants planted in 1950 and 1951 passed through the process of development 10 and 16 days more rapidly than in 1949. It follows from this that drought for summer grasses results in an acceleration of passing through the stages of development. A similar influence in a series of years, as we will see below, leads to a strengthening of the new characteristic (in our case, the acceleration of the rhythm of development).

TABLE 3

Structure of the Harvest

Reproduction	Number of spikes per ear	Number of grains per ear	Weight of 1,000 grains, g	Productive sets, %
1949				
Alma-Atinskaya	11.5	20.54	30.25	0.74
Dzhezkazganskaya	10.0	20.01	29.86	0.78
Grekum 1538	9.8	19.5	28.81	0.78
1950				
Saratovskaya	9.8	19.2	26.654	0.66
Dzhezkazganskaya	9.5	17.4	25.341	0.67
1951				
Saratovskaya	10.2	20.12	27.318	0.64
Dzhezkazganskaya	10.12	18.94	26.715	0.67
Karagandinskaya	10.58	20.28	29.783	0.75

The marked changes in the rate of progress of the development in relation to the conditions of the year reflect only the normal picture of the reactions of the plants to the influence of drought or the directions of this reaction. We cite the data that we obtained for an answer to the question: to what degree are the changes in the rhythm of development carried on under the effect of the influence of many years of dry weather and are these changes strengthened?

The calculation of the number of plants developing spikes and flowering made from the moment of the start of spike development up to the period when 50% of all the plants (stems) in the row flowered, shows the earlier start of spike development and flowering of the Dzhezkazganskaya reproduction in comparison to all of the others. The individual plants began to develop spikes earlier than the Alma-Atinskaya by one to two days in the experiments in 1949 for the Dzhezkazganskaya reproduction and the acceleration in the start of the flowering of the basic mass was three to four days. The difference increased still more in the period of ripening: the Dzhezkazganskaya ripened five to six days earlier than the Alma-Atinskaya. The

difference between the Dzhezkazganskaya and the Grekum, according to the ordinary mass of flowering plants, was three to four days and according to the beginning of ripening, five to six days. The degree of differences in the rate of passing through the process of development between the reproductions and the Grekum type were practically of the same order. This suggests that the changes appearing in the tempo of development for the Dzhezkazganskaya reproduction are located in the range of variety differences. A comparison of the Dzhezkazganskaya and Alma-Atinskaya reproductions positively cannot give a reliable expression of how far the first wheat changes under the effect of new conditions. For this it would be necessary to compare them with the maternal variety . The results of the investigations carried out in this connection in 1950 and 1951 show that the Dzhezkazganskaya reproduction actually varies significantly in the rate of development from the original (maternal) variety. According to the data in 1950, the main mass of the plants of the Dzhezkazganskaya wheat flowered on June 13, and of the Saratovskaya only on June 16, the difference being three to four days. According to the data of 1951, these differences were four to five days on the side of a more rapid beginning of flowering for the Dzhezhazganskaya wheat. The Karagandinskaya (half) reproduction delayed flowering one to two days in comparison with the Saratovskaya, and in comparison with the Dzhezkazganskaya, five to six days. The Dzhezkazganskaya reproduction varies significantly from the Saratovskaya in the time of the start of the period of full ripeness of the seeds. The Dzhezkazganskaya reproduction ripened faster than the Saratovskaya by five to six days in the experiments in 1950 and 1951, and compared to the Alma-Atinskaya and Karagandinskaya, respectively five to six and seven to eight days.

The accelerated development of the Dzhezkazganskaya reproduction arising as a result of the many years of cultivation in desert conditions is analagous to the above indicated normal characteristic of this variety of wheat for a more rapid passing through of its cycle of development in the drier years, and also to a later date of planting in the given year. All of this suggests that the inadequate water content of the soil in conjunction with atmospheric dryness inevitably leads to somewhat of an acceleration in passing through the development. The latter undoubtedly is a measure of the reaction of the plant organism to drought, a reaction directed at the adjustment of the rhythm of development in accordance with the new conditions of the environment.

A peculiarity of the rhythm of development of the Dzhezkazganskaya reproduction is the shorter time for spike development, flowering and ripening. To a greater extent its adaptation shows more sprouts than for other reproductions (relatively thin sprouts were noted for the Saratovskaya and Karagandinskaya reproductions). Thus, from the point of view of the change in the rhythm of development, the adaptation to drought of this type of wheat cannot be looked upon without a connection with the rate of development. As for short-lived vegetation, its adaptation to drought is determined to a significant degree by the rate of completion of the cycle of development in the relatively unfavorable period in the conditions of the desert (from the point of view of soil moisture). In connection with this, the completely correct assertion of several authors [6, 8] on the known role of the rate of development in the determination of the characteristics of drought resistance of summer grasses is presented.

The accelerated tempo of development of the Dzhezkazganskaya reproduction, as a result of many years influence of desert conditions, can be reasonably viewed as a newly acquired characteristic. Being a reaction of adaptation, this rhythm of development must at the same time be viewed as being progressive. At the same time, the differences between the reproductions according to this characteristic, and separated by the limits of the variety, make it possible to consider this characteristic as strengthening the genetic basis of the variety.

The shifts in growth and development, on which the varieties are based, originating under the effect of the conditions of the environment, to which are added the characteristics of the metabolism of substances, are inclined to change themselves in the morphological structure. It is known that an insufficient water supply results in a decrease in the size of the leaf surface [11]. The measurements of the area of the leaf blade that we carried out for three stages of the different reproductions showed (Table 1) less development for the Dzhezkazganskaya reproduction cultivated in the desert than for the normal leaf surface.

The average leaf area appeared to have the greatest development for the Karagandinskaya reproduction; Saratovskaya occupied the middle position. The length of the leaf blades in sum for the three levels was highest for the Dzhezkazganskaya reproduction, the width, on the other hand, for Saratovskaya. Therefore the size of the leaves changed in the direction of increasing their length and decreasing their width for the Dzhezkazganskaya reproduction under the effect of severe desert conditions. This form of the leaves and also their sizes, it appears, is more favorable from the point of view of economy of loss of water to evaporation, and, at the same time, is a newly acquired feature.

It is of interest to follow the relationship between the changes in the rate of development and growth processes for the reproductions as developed under the effect of many years of drought action. The results given in Table 2 show the essential lag in the growth of the Dzhezkazhanskaya reproduction from the other investigations. The lag of the growth processes begins in the period of stem formation; on the other hand, it grows noticeably faster than Saratovskaya in the period of sprouting.

An insufficient water supply involves a retardation of the growth processes and the formation, in this connection, of a small-celled condition [7-12]. The retarding of the growth processes under the effect of drought was shown in a series of works [13-15]. At the same time, according to the data of these authors, the process adapted to the insufficient water supply, accompanied by a stop in the growth processes, leads to a reduction in the productivity. The same reason, the inadequate water supply, influencing the growth processes from year to year by slowing them, leads to the change in the tempo of growth for the Dzhezkazganskaya reproduction. In the given case interest is presented for us not by the fact of the retardation of the growth processes itself under the effect of drought, but by the relationship existing between the characteristic of growth and the development in ontogenesis. Viewing growth and development in their interconnection, it can be proved that the slow growth accompanied by fast development is a biological feature lying at the base of the drought resistance of summer wheat Eritrospermum 841.

The cumulative change in rhythm of the growth and development for the Dzhezkazganskaya reproduction in accordance with the new conditions of the environment could not lead to the corresponding changes in productivity. The data given in Table 3 show that the Dzhezkazganskaya reproduction, in comparison with all of the remaining reproductions has lower quantities of the elements that constitute the structure of the harvest. The lower activity in the accumulation of dry substances (Table 2) also corresponds to this.

Confirmation of higher productivity for the Dzhezkazganskaya reproduction in comparison to that for Alma-Atinskaya as established in the experiments of other authors [16], was not found in our investigations. It must be noted that all of the reproductions studied are drought resistant to a greater or lesser extent. The Dzhezkazganskaya reproduction, in relation to the others, contributed to some instability of the variety under the influence of many years of unfavorable conditions. At the same time the small advantage of the Dzhezkazganskaya reproduction in the character of the productivity of the plants could not be noted.

Thus, the new rhythm of development and the growth connected with it acquired by the Dzhezkazganskaya reproduction correspond to the level of the harvest, to the indicated adaptation to the given level of the intensity of meterorological factors and to the concentration of water in the soil.

SUMMARY

1. Many years of cultivation of soft summer wheat in very dry conditions lead to an acceleration of development and retardation of growth, an increase in the length of the leaf blades and a decrease in their width and average leaf surface area.

2. Changes in the tempo of development and growth under the effect of an inadequate water supply is indicated by the degree of reaction of the plant to drought and represents one of the essential sides of the biological cause of drought resistance of plant organisms.

3. Shortening of the period of vegetation for the Dzhezkazganskaya reproduction took place in proportion to the shortening of the period prior to spike development, as well as ripening.

4. The differences in the tempo, the rhythm of development and the growth between the Dzhezkazganskaya reproduction and the Saratovskaya (maternal) lie within the limits of variety differences.

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SOME FEATURES OF THE ASSIMILATION OF SUBSTANCES THROUGH THE LEAVES AFTER FOLIAR APPLICATION

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We know a priori that the process of assimilation of substances through the leaves must proceed in a different manner than the intake of substances through the roots because of the anatomical features of the leaf construction, in part, the presence of the cuticle. Van Overbeek [1] thought that the dry cuticle was in general impermeable to water and substances soluble in water. However under the action of water, over a period of time, the cuticle swelled, and its impermeability was broken. If this is actually so, then the assimilation of substances in the first moments after placing a solution on the surface of the leaf can be absent, or in any case, is very small quantitatively.

However, if it is thought that the nutrient solution dries very quickly after being placed on the leaves, then, in general, it is not clear how, when, and under what conditions the process of the assimilation of substances through the leaves takes place.

The information in the literature on the theory of foliar application of nutrients for plants is very insignificant and to some extent contradictory. Besides this, the correct answer to this question has not only theoretical, but also great practical significance for carrying out foliar nutrient application in plants, interest in which is growing more and more in domestic and foreign research and practical agriculture. It is enough to show that for the last two to three years in the Russian language alone more than two hundred articles by Soviet and foreign authors in the form of original work and papers have been published. They devoted in the main part to the effect of foliar nutrient application on the harvest. The necessity for a theoretical treatment of the question of the assimilation of substances through the leaves of plants can be understood in connection with this.

The results of research on the study of the kinetics of the assimilation of P^{32}, Ca^{45} and S^{35} through the leaf, the effect of pH and the associated cations on the assimilation of P^{32} from solutions of different concentrations, and also several questions on the movement of P^{32} in the plant are given in this article.

METHOD

We studied the assimilation of substances after foliar nutrient application using radioactive isotopes P^{32} Ca^{45} and S^{35} by calculating the impulses in conjunction with the method of radioautography in a series of experiments. Radioactive compounds in the form of solutions with concentrations of 0.1 to 0.006 M with a unit activity of approximately 10 μC/ml were placed in 0.02 ml drops on growing leaves on the upper parts of plants of tomato, cucumber, primrose and lettuce. From 5 to 20 drops of the solution were placed on the plants. The compounds not entering the leaves were washed from the surface of the leaf with a jet of water from a water faucet for a period of two minutes at the completion of the experiment. As the control experiments showed, this time was fully adequate for the practically complete removal from the surface of the leaf of the isotope which did not enter into the leaf. The assimilation of P^{32} from solutions of singly and doubly replaced phosphates of Na, K, NH_4 and their buffers, and also from solutions of Ca $(H_2PO_4)_2$ and H_2PO_4, was studied in this work. The

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concentration of the solutions of compounds of phosphorus varied from 0.1 to 0.006 M. The assimilation of Ca^{45} through the leaves was studied using a solution $CaCl_2$ and S^{35} using a solution of Na_2SO_4.

The entire plant, including the roots, was analyzed for radioactivity in the experiments on the investigation of the effect of pH and the associated cations on the assimilation of P^{32}, and also in the experiments on the effect of repeated washing on the assimilations of P^{32}. Because the goal of the experiments on the investigation of the kinetics of the assimilation of P^{32}, Ca^{45} and S^{35}, in essence, consisted of the determination of the time that the isotopes began to be assimilated into the leaves, in these experiments we determined the radioactivity only for the leaves on which the isotopes had been placed (experimental leaves) without measuring the

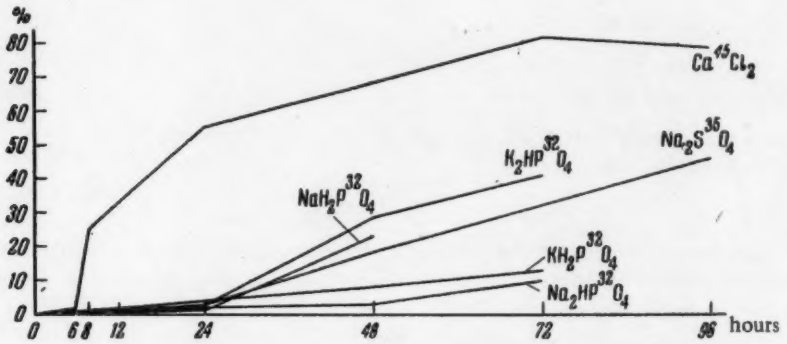

Fig. 1. Kinetics of the assimilation of salts into the leaves of tomatoes.

outflow of isotopes from these leaves. As we will show below, such a method of measuring the assimilation of isotopes for tomatoes gives completely comparable relative data because approximately 100 to 75% of the P^{32} of the normal activity of the plant remains in the experimental leaves even after three days. Therefore, calculating the assimilation of P^{32} by its concentration in the experimental leaves was used in other of our experiments. The calculation of the impulses was carried out in the type "B" apparatus in standard conditions by the standard method. Readings of the radioautograph were obtained on isopanchromatic film used in the photographs. In the tables shown below, the absorption of radiosotopes by the leaves of plants is given as the percent of the dose placed on the leaf, and the quantity flowing out of the experimental leaves as the percent based on the normal radioactivity of the plant.

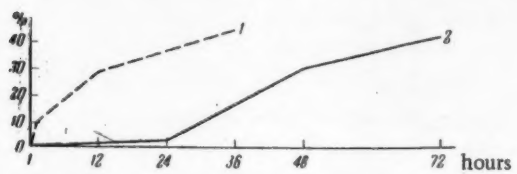

Fig. 2. Kinetics of the assimilation of P^{32} into the leaves of tomatoes from $K_2HP^{32}O_4$. 1) on rupturing the surface of the leaf. 2) without rupturing.

Fig. 3. Kinetics of the assimilation of P^{32} from $K_2HP^{32}O_4$ on rupturing the surface of the leaves of cucumber (1) and tomato (2).

Results of Investigations of the Kinetics of the Assimilation of Substances into the Leaves of Plants

Kinetic curves are shown in the graph (Fig. 1) for the intake through the leaves of tomato of $Ca^{45}Cl_2$, marked phosphates and $Na_2S^{35}O_4$ from a solution of 0.1 M concentration. We see from the graph that Ca^{45} from calcium chloride is characterized by a greater rate of assimilation into the leaf than P^{32} from marked phosphate and S^{35} from sodium sulfate. This brings to our attention the fact that the process of assimilation of Ca^{45} into the leaf begins not immediately after placing drops of the solution on the leaf, but after approximately 4 to 6

hours. The time from the moment when drops of the salt solution are placed on the leaf up to the start of its assimilation into the leaf, will be termed the "latent phase of assimilation". The curve for the assimilation of Ca^{45} turns up abruptly after the latent phase so that in 24 hours up to 56% of the Ca^{45} placed on the leaf has already entered, and after 72 hours, up to 80% of the Ca^{45} has entered.

The time of the latent phase for phosphates and sulfate in the experiment was equal for approximately 24 hours. At this time 1.4 to 1.8% of the salts had gone into the leaves. Only on the second day did the visible process of assimilation of salts begin, whereupon the rates of assimilation of phosphates were sharply differentiated. After the second day, the assimilation of P^{32} from K_2HPO_4 was 29.4%, but from Na_2PO_4 only 2.8%; the assimilation of P^{32} from NaH_2PO_4 was 23.7%, but from KH_2PO_4 only 7.6%. After the third day the assimilation of P^{32} from K_2HPO_4 was 41.5%, but from Na_2HPO_4 only 10.0%. Thus K_2HPO_4 has the highest rate of assimilation and Na_2HPO_4 the lowest for phosphates.

The assimilation of S^{35} after the second day was 19.7% and after the fourth, 46.3%. The curve for the assimilation of sulfate is located inside the family of curves for the assimilation of phosphates.

The additional experiment with cucumbers showed that the character of the assimilation of $Ca^{45}Cl_2$ through the leaves of cucumber suggests the character of the assimilation through the leaves of tomato. However the assimilation of $Na_2S^{35}O_4$ through the leaves of cucumber even after 120 hours is expressed very weakly (Table 1).

We will attempt to give some explanation for the fact noted above. Based on the ideas of Overbeek [1], we are inclined to explain the presence of a latent phase in the assimilation of substanced through the leaves by the existence of a cuticle on the surface of the leaf which impedes the assimilation of substances. This proposition also suggests experiments with light mechanical rupture of the surface of the leaf. The latent phase is absent when the surface of the leaf is ruptured by light rubbing with a glass rod, and the assimilation of salts begins the moment the solution is placed on the leaf (Fig. 2,3). It must be stated that the inhibiting effect of the cuticle on the rate of assimilation of isotopes was not noted in the work of Bucovac and Wittwer [2], in which the assimilation of a series of isotopes through the leaves of bean shoots was studied. Possibly this fact can be explained by the fact that the cuticle is poorly developed for bean shoots, but the authors explain that relatively long periods of time from the moment of the beginning of the experiment were taken for the observation of the kinetic assimilation of isotopes.

TABLE 1

Assimilation of C^{45} and S^{35} into the Leaves of Cucumber

Time, hours	Impulse/minute	%	Time, hours	Impulse, minute	%
Assimilation of Ca^{45}			Assimilation of S^{35}		
4	1120	6.9	24	40	0.0
8	7018	43.0	48	105	0.8
			96	200	1.7
24	7528	46.0	120	204	1.7

Conditions of the Assimilation of Substances Through the Leaves after Foliar Nutrient Application

If the experiment is carried out on leaves for which the cuticle is well developed, then the latent phase can measure many hours. After the time of the latent phase, the drops of the solution placed on the surface of the leaf dry, and most of the salts form crystals forming a precipitate. However, the process of assimilation of salts does not stop after formation of a precipitate, but, on the contrary, begins as soon as before. We explain this phenomenon by the fact that the salts do not go completely into the precipitate, but partially remain on the leaf in the form of a saturated solution to the degree of the attraction of moisture from the air. The practical assimilation of substances into the leaf also takes place from the water film of the saturated solution. In connection with the indicated, we can expect that if the given salt is capable of being held in a saturated solution to a large degree and does not form a precipitate, then this salt will move through the leaf in large quantities. The ability of the salts to maintain the condition of a saturated solution for a long

TABLE 2

The Effect of Repeated Wetting on the Assimilation of P^{32} from $K_2HP^{32}O_4$ in Primrose (%)

Precipitate	Without wetting	With wetting
Yes	24.1	55.4
No	42.9	40.6

Note. The duration of the experiment (t) was three days.

TABLE 3

The Assimilation of P^{32} Through the Leaves of Tomato

Radioactivity as % of dose	KH_2PO_4	KH_2PO_4 + K_2HPO_4	K_2HPO_4	NaH_2PO_4	NaH_2PO_4 + Na_2HPO_4	Na_2HPO_4	$NH_4H_2PO_4$	$NH_4H_2PO_4$ + $(NH_4)_2HPO_4$	$(NH_4)_2HPO_4$
Entire plant	54.5	74.4	81.5	66.8	44.4	28.0	64.2	47.7	60.2
Experimental leaves	44.6	55.3	63.0	64.6	40.1	23.5	55.1	42.6	52.3

Note. The duration of the experiment was three days. The concentration of the solutions placed on the leaf was 0.006 M.

time is connected with their solubility and hygroscopity. We explain the more rapid assimilation of $CaCl_2$ and K_2HPO_4, when compared to other salts, by the high solubility and hygroscopity of the indicated salts, which, in the optimal conditions of air moisture, absolutely do not precipitate after the evaporation of water of the nutrient solution from the surface of the leaves, while at the same time Na_2SO_4, Na_2HPO_4, KH_2PO_4 and $Ca(H_2PO_4)_2$ all quickly form a precipitate. These compounds that are inclined to form a precipitate were also characterized in the experiment described above by a weakly expressed assimilation into the leaf.

TABLE 4

Assimilation of P^{32} into the Leaves of Tomato from Solutions with Different Concentrations

Concentration of the solution	Hours	KH_2PO_4	K_2HOP_4	$NH_4H_2PO_4$	$(NH_4)_2HPO_4$	$Ca(H_2PO_4)_2$	H_3PO_4	NaH_2PO_4	Na_2HPO_4
0.1 M	12	0.4	1.4	–	–	–	34.6	2.1	0.2
0.006 M	24	24.2	12.6	23.3	17.6	18.8	18.6	–	–
	48	18.0	65.8	38.9	45.0	23.2	14.8	–	–
	72	63.3	72.1	41.3	65.2	37.2	43.5	–	–
0.02 M	72	37.2	62.2	33.2	39.1	–	47.7	50.8	13.6

Note. Outflow not determined.

The transformation of salts into the precipitate after the evaporation of water or their ability to maintain a condition of saturated solution depends in many cases also on the relative humidity of the air. Thus, for example, hygroscopic K_2HPO_4 can for a low relative humidity of the air, forma precipitate, the repeated dissolving of which by means of wetting the experimental leaves with water, aids in the additional assimilation of P^{32}. With a high relative humidity of the air, K_2HPO_4 does not form a precipitate and sprinkling the experimental leaves with water has no effect (Table 2).

The condition of the saturated solution, from which the assimilation of substances through the leaves takes place, determines the character of the relationship between the process of assimilation and several factors, in part the pH level and the associated cations.

The Effect of pH and the Accompanying Cations on the Assimilation of P^{32} through the Leaves

The hypothesis that with foliar nutrient application, the solubility and hygroscopity of the phosphates, shows a decided effect on the rate of intake of salt, is confirmed by the example of the study of the assimilation

of P^{32} from acid and alkaline phosphates and their buffers. It is seen graphically from these data, which were shown above, that the rules for the assimilation of phosphorus through the leaves from acid and alkaline solutions by no means always agrees with the rules established for the assimilation of phosphate ions through the roots [3,4]. Actually, the assimilation from an alkaline solution proceeds significantly slower than that from an acid solution in the case of sodium phosphate (Table 3) and this agrees with the case that was observed on the absorption of phosphate ions by the roots. We have an opposite picture in the case of the assimilation of P^{32} from a solution of sodium phosphate: the assimilation of P^{32} from the alkaline phosphate proceeds significantly more energetically than from an acid solution, that is, the opposite of the case observed for the absorption of sodium phosphate through the roots [4].

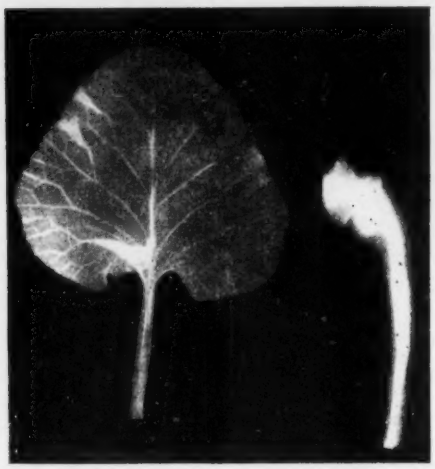

Fig. 4. Radioautograph of a young leaf and developing flower of primrose. A radioautograph of the remaining parts of the plant was not obtained because of the lower concentration of P^{32} in them.

It is quite obvious that the differences that we discovered in the assimilation of P^{32} from various phosphates through the leaves cannot be explained by the ideas borrowed from the established rules for the effect of pH on the assimilation of phosphate ions through the roots. These data could be better explained by the determination of the role and importance of the solubility and hygroscopity of salts for their assimilation through the leaves after foliar nutrient application. Actually the solubility of phosphates of potassium with an increase in their pH from 4.5 to 9 increases, and for phosphates of sodium decreases (see Chemistry Reference). Corresponding to this, the assimilation of P^{32} increases from phosphates of potassium but decreases from phosphates of sodium to the extent of the increase of pH on transfer from a phosphate acid to an alkaline.

We did not discover a constant rule for the character of the effect of pH on the assimilation of P^{32} for phosphorus of ammonia (Table 3), but the solubility of the alkaline phosphate of ammonia was higher than for the acid. Therefore we must anticipate a more energetic assimilation of P^{32} from the alkaline phosphate of ammonia than from the acid; that also has a place in most of our experiments (Table 4) and also in the experiments of Eggert, Kardos and Smit [5]. We can see graphically from Table 3 that the character of the rule on the

TABLE 5

Movement of P^{32} from the Experimental Leaves of Tomato (% of normal radioactivity of the plant)

Parts of the Plant	KH_2PO_4		KH_2PO_4 + $+K_2HPO_4$		K_2HPO_4		NaH_2PO_4		NaH_2PO_4 + $+Na_2HPO_4$		Na_2HPO_4	
	1	2	1	2	1	2	1	2	1	2	1	2
Aerial portion	16.1	11.5	21.4	12.4	18.8	4.3	2.5	3.7	7.5	4.5	12.7	2.0
Roots	2.2	0.7	4.2	0.4	3.8	0.0	0.7	0.0	1.5	0.5	3.1	0.0
Total outflow	18.3	12.2	25.6	12.8	22.6	4.3	3.2	3.7	9.0	6.7	15.8	2.0

Note: The outflow is equal to the sum of the radioactivity of the aerial portion (less the experimental leaves) plus the radioactivity of the roots; figures 1 and 2 in the table heading indicate the experiment number. The first experiment was carried out under favorable temperature conditions; the second, with a significant decrease in the air temperature.

effect of pH on the assimilates of P^{32}, as we discovered, survives to the extent of the assimilation of P^{32} according to its concentration not in the entire plant, but only in the experimental leaves.

There is no mention in the literature of the character of the effect of pH on the assimilation of substances through the leaves. According to Matskov [6], acidifying the medium increases the assimilation of phosphorus and alkalization, on the other hand, decreases the assimilation. Swanson and Whitney [7] hold to such a view. Tukey, Wittwer, Teubner and Long [8] think that the assimilation of phosphorus depends on the pH of the solution; however, their experimental data suggest that with an increase in pH from 4 to 9 the assimilation of P^{32} from phosphates of sodium actually decreases, but on the other hand, increases from phosphates of potassium.

The point of view that we take on the decided effect of hygroscopity and solubility of the salts on their assimilation through the leaves, covering the effect of pH, gives support to the work of Koontz and Biddulph [9], which appeared recently. On the basis of experiments with P^{32}, these authors also came to the conclusion that the quantity of absorption and transfer of P^{32} in the plants does not depend on the pH of the solution placed on the leaves, but depends on the capacity of the salts to hold moisture, that is, on their hygroscopity.

We also looked at the effect of the valence of the accompanying cations on the assimilation of phosphorus in relation to the nature of the solubility of the salts. Thus, for example, the weak assimilation of P^{32} from $Ca(H_2PO_4)_2$ can be explained only by the low solubility of this phosphate (Table 4).

It must be stressed, however, that the solubility of compounds by no means is the only factor determining the role of assimilation of P^{32} through the leaves. Certainly a large role is also played by factors of a physiological order in this phenomenon. This is confirmed by experiments on the study of the assimilation of free phosphoric acid in comparison with the phosphates on a background of different concentrations of solution. H_3PO_4 enters more energetically than other phosphates with a high concentration (0.1 M). However, it is not difficult to notice that the H_3PO_4 enters more slowly than many phosphates with a lower concentration of phosphate compounds to 0.02 M and especially to 0.006 M. (Table 4).

Obviously the slow assimilation of H_3PO_4 from solutions of hydrogen shows a negative physiological action on protoplasm, in answer to which the protoplasm actively inhibits the assimilation of the acid. With high concentrations, H_3PO_4 quickly attacks the cutin, destroys the protoplasm (that is confirmed by the presence of strong burns) and without hindrance passes through into the leaf. Thus the data on the features of the assimilation of phosphoric acid also confirm the position that we take on the lack of a relationship of the assimilation of phosphorus after foliar application to the pH of the solution. It follows from the above that it is necessary to take those concentrations that do not cause burns on the leaves when studying the assimilation of substances throught the leaves. In our experiments the safe concentration of compounds of phosphorus was slightly lower than concentration of 0.006 M. However, in our experiments we used a concentration of 0.006 M as the least dangerous for the leaves because work with concentrations lower than 0.006 M presents several methodological difficulties. The safe concentration for other compounds could be higher. Thus for example, $CaCl_2$ even in a concentration of 0.1 M did not cause burning of the leaves for study plants.

The Movement and Distribution of P^{32} in Plants after Foliar Application

The P^{32} outflow from the experimental leaves in general is directed into the growing aerial organs of the plant (Fig. 4), and to the roots in a very insiginificant quantity, as we discovered for tomato, cucumber and primrose . Based on the work of Koontz and Biddulph [9], we explain the weak movement of P^{32} in the roots by the fact that the isotope in our experiments was placed on leaves in the upper stage of the plants. A greater quantity of P^{32} remained in the experimental leaves, generally in the place where the isotopes were placed on the leaf; therefore, the data of several authors [10], which do not consider the quantity of P^{32} remaining on the place where the drops of the solution with the isotope were placed on the leaf, are in need of serious correcting from our point of view.

The translocation of P^{32} from the experimental leaves into the plant takes place relatively slowly, so that after three days the amount of the outflow of P^{32} varies for tomato approximately from 2.0 to 25% and for cucumber from 10 to 30%. The outflow was 2.4 to 9.0% of the normal radioactivity of the plant for primrose in the winter and early spring period. The outflow of P^{32} takes place more energetically for lettuce and varies in the range of 37 to 57%. We took the lettuce for this experiment in the period of intense growth,and this probably explains the higher figures characterizing the movement of P^{32}. The movement of P^{32} from the

experimental leaves is aided to a larger extent by the cation of potassium than the cations of sodium or ammonia (Tables 5 and 6). The cation of ammonia, in its turn, more strongly affects the movement of P^{32} from the experimental leaves of lettuce than do the cations of potassium and hydrogen, as we can see from the following data:

Compound	$NH_4H_2PO_4$	$(NH_4)_2HPO_4$	$Ca(H_2PO_4)_2$	H_3PO_4
Outflow	57.0	43.0	37.0	40.0

The temperature factor also has an effect on the intensity of the outlfow of P^{32}. The outflow of P^{32} is sharply lowered (Table 5, experiment 2) when the temperature is lowered in the greenhouse at night and in the morning (to 11-13° at 8 A. M. hours in the morning) in spite of the presence of an optimal temperature during the day (26-30°). It is interesting to notice that these fluctuations of the temperature, which took place in experiment 2, were not reflected in the assimilation of P^{32}. On the other hand, in the variant with K_2HPO_4 the normal assimilation of P^{32} even approached the maximum and equaled 100%, while at the same time the outflow was only 4.3%. The outflow of P^{32} was still less in the variants with NaH_2PO_4 and Na_2HPO_4 of experiment 2.

TABLE 6

Movement of P^{32} from Experimental Leaves of Tomato

Part of the plant	KH_2PO_4	KH_2PO_4 + K_2HPO_4	K_2HPO_4	$NH_4H_2PO_4$	$NH_4H_2PO_4$ + $(NH_4)_2HPO_4$	$(NH_4)_2HPO_4$
Aerial portion	20.4	14.8	4.6	11.9	8.9	9.0
Roots	2.2	0.5	0.5	2.2	1.8	4.1
Total outflow	22.6	15.3	5.1	14.1	10.7	13.1

SUMMARY

There is a latent phase (no assimilation during the beginning period of time) in the assimilation of mineral substances through the leaves. It was established in the present work with marked compounds of potassium, phosphorus and sulfur that the duration of the latent phase depends on the properties of the substances placed on the leaves and on the species characteristics of the experimental plants. The latent phase can last for many hours in plants with a developed cuticle.

Different substances enter through the leaves at different rates, for which some of the deciding factors are the hygroscopity and solubility of the salts. In the case of their solubility of different bases, the importance of their solubility is superimposed on the importance of their pH. Besides the factor of the solubility of the salts, factors of a physiological nature, in part the selective characteristics of the protoplasm in relation to the absorption of ions, also show an effect on their assimilation into the leaf.

The outflow of P^{32} is subjected to a sharp fluctuation in relation to the physiological condition of the plant and the above factors also make up only a part of the normal quantity of P^{32} assimilated into the plant. The outflow of P^{32} averaged 10-20% of the normal assimilation of phosphorus into the plant even after three days under experimental conditions after placing a solution on the upper surface of young leaves of tomato. From the accompanying cations, the greatest effect on the movement of P^{32} is shown by potassium. The fluctuation of the temperature from 26-30° during the day to 11-13° in the morning is strongly reflected in the outflow of P^{32} but does not show any effect on its assimilation.

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THE EFFECT OF PREPLANTING HARDENING OF SEEDS AGAINST DROUGHT ON THE GRAIN HARVEST OF CORN IN LATE PLANTINGS

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In Kuban there are more than one hundred days between the harvest of grains and the time of frost. Such a period of time is fully adequate for a second harvest of several crops by late planting. The largest increase in late plantings is obtained from corn.

It appears possible to definitely obtain a harvest both of vegetation and grain by planting corn.

However, obtaining a high harvest of corn in late planting is possible only in years of adequate moisture, which do not often occur in Kuban. The level part of the Krasnodar region lies in a zone of inadequate moisture where, during the period of growth of late-planted crops, there are droughts almost every summer that not only lower the harvest of corn, but also cause total mortality of plantings in some cases.

In order to increase the drought resistance of corn in late plantings, we used the method of preplanting hardening of seeds against drought developed by Genkel' [1].

The experimental work was carried out in 1956 and 1957 on the scientific-experimental form of the Kuban Agricultural Institute. There were two repetitions using paired plots of 200 square meters each. In the years of the investigations on the growth of corn, the same agrotechnical method was used.

We used different means of hardening the seeds in contrast to the method used by Demina [2]. This was because we carried out the hardening at the end of June when the air temperature was fairly high (average 25 to 27°). The seeds swelled quickly under these conditions and 24 hours after soaking more than 70% of them had an easily discernible embryo under the seed coat. For many of the seeds at this time the radicles emerged through the seed coat. A longer period of soaking resulted in the sprouting of most of the seeds, as a result of which their later germination decreased. Following this, the seeds were dried in the air as soon as possible, and they again took on their normal weight after approximately 24 hours and 2 to 3 stirrings.

The variants of preplanting treatments of seed that we studied and also the results of the observation of their field germination are given in Table 1.

As we can see from Table 1, the hardening of the seeds increased the field germination of corn [3]. As a result of this, the number of empty sets on a planted plot was decreased, and the number of sets with emerging sprouts correspondingly increased.

However the increase in germination was observed only in those variants where the number of sprouting seeds at the time of hardening was lowest. With an increase in the length of time of the wetting the number of sprouting seeds increased and their field germination decreased as a result of the hardening. This can explain the decrease in the field germination and harvest of corn in those variants in which the seeds sprouted (double soaking for 24 hours with the emergence of the radicles in 1956, and soaking for 36 hours in 1957). The greatest loss took place in those variants in which soaking was continued for a longer period (a single stage of 48 hours and two stages of 36 and 48 hours). This was the reason for dropping these from the experiment.

TABLE 1

Field Germination of Seed and Number of Sets with Emerging Sprouts (as % of the control)

Means of hardening seeds	1956		1957	
	field germination	number of sets with plants	field germination	number of sets with plants
Air-dry seeds (control	100	100	100	100
Soaked 15 hours (without drying)	122	115	103	105
Soaked 24 hours	111	106	105	109
Two stage, soaked for 24 hours	122	113	100	94
The same (radicles emerged from the seed coat)	72	95	–	–
Soaked 36 hours	–	–	95	87
Two stage, soaked for 36 hours	–	–	35	24
Soaked 48 hours	–	–	87	72
Two stage, soaked for 48 hours	–	–	20	11

TABLE 2

The Effect of Hardening Seeds on the Harvest of Corn

Means of hardening seeds	1956		1957			
	harvest of green vegetation, centners/hectare	harvest as % of control	normal harvest of green vegetation, centners/hectare	harvest as % of control	harvest of cobs in milk stage, centners/hectare	harvest of air-dry substance, centners/hectare
Air-dry seeds	16.2	100	170.7	100	44.7	44.4
Soaking 15 hours (without drying)	27.2	169	178.0	104	50.9	46.3
Soaking 24 hours	20.8	128	199.7	117	54.2	52.0
Two stage, soaking for 24 hours	22.8	140	204.0	119	59.4	53.4
Same (radicles emerged from the seed coats)	19.9	123	–	–	–	–
Soaking 36 hours	–	–	170.4	100	43.4	44.3

The harvest of corn increased as a result of the hardening of seeds. In our experiments it increased up to 19% under conditions of sufficient moisture (1957), and with insufficient soil moisture (1956), up to 40%. Together with the increase in the normal harvest there were increases both in the harvest of cobs and in the accumulation of dry substance (Table 2).

The increase in field germination observed in the experiments and also the increase in the size and weight of the plant indicates an increase in the drought resistance of corn as a result of hardening of the seeds [4].

There was also a relative increase of the reproductive organs (cobs) as part of the harvest in 1957 connected with the preplanting hardening of seeds, as can be seen from the following data (in %):

Means of hardening seeds	Leaves	Stems	Cobs	Husks
Air-dried seeds (control)	28	40	14	18
Soaked 15 hours (without drying)	27	41	14	18
Soaked 24 hours	26	42	16	16
Two stage, soaking for 24 hours	25	42	17	16
Soaked 36 hours	26	42	13	19

Positive results from the planting of soaked weeds (without drying) were observed in 1956 when the planting of corn was carried out after rain. In this case sprouts of corn appeared two to three days early, using moisture resulting from the rain to a large extent, and grew significantly better up to the time of drought. In 1957 the seeds that had been hardened had the advantage. In addition to this, planting soaked seeds in a soil which had insufficient moisture could be a reason for their death that was eliminated by planting hardened seeds.

SUMMARY

1. Preplanting hardening of seeds by the P. A. Genkel' method shows a positive effect on the growth and development of late planted corn, and also on its harvest.

2. The most effective means of hardening are single and double soakings of the seeds (24 hours) followed by drying after each soaking to an air-dry condition.

3. Corn seeds which sprouted during the process of hardening had a lower germination. In connection with this it is necessary that the drying of soaked seeds begins when there is a clearly defined embryo in the majority of the seeds, but the emergence of the radicle through the seed coat must not be permitted.

4. The method of preplanting hardening of seeds could be recommended for wide use in crop production in summer (at harvest and later) planting of corn when growing it in conditions of inadequate moisture without sprinkling, and also with irrigation.

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FORMATION OF LIGNIFIED CELL WALLS

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The study of the chemical nature and physical properties of vegetative membranes, and elucidation of the biosynthetic paths of their components in living plants, are very important to the successful resolution of different practical problems related to the chemical treatment of vegetative raw material as well as to individual problems of plant physiology. To these belong, first of all, studies of the effects of different climatic conditions and, in particular, the effect of low temperatures on woody tissue ripening, which conditions the specific mechanical sturdiness of plants, as well as their biological resistance, to a certain extent. Elucidation of the fine structure of cell walls and of growth changes in their chemical composition is important also in studying such basic problems of plant physiology as their mineral nutrition.

D. A. Sabinin indicated that the study of mineral nutruition should begin with reactions which occur on surfaces of vegetative cells with the ions of the external medium [1]. In such cases "the topographic point of these interactions is where the reactions occur on the membranes of vegetative cells." Therefore, it can be assumed that changes in properties of the membrane on aging will also change the character of these primary interactions.

The volume occupied by cell walls is very large in the vegetative organism. Suffice it to say that in the woody varieties the main high molecular weight components of membranes – cellulose, hemicellulose, and lignin – comprise up to 90-95% of the dry weight of woody tissue [2]. During the plant's life activity the chemical composition of cell membranes and their physicochemical properties change. According to Paleev's [3] data, changes in the quantitative relationship of the cell-wall components, observed at the time of straw formation, are at the base of wilt phenomena of grain cultures, which result in much damage to agricultural crops. At this point it should be noted that changes in the chemical composition of cell walls with age are evidently also related to changes in the inner structure of the membrane components themselves. Thus, according to Odintsov's [4] data, changes of the inner structure in aged cellulose and cholocellulose lead to a decrease in bound water, which depends upon the formation of hydrogen bonds with previously hydrated hydroxyls. Therefore, for a study of formation and subsequent change of membranes in general, it is insufficient to explain the biosynthetic paths of the individual components; an explanation is also necessary for the interactions at different stages of their formation.

Evidently, precisely these definite interrelationships which are combined in the developing cell-wall between its changing components also determine that combination of properties which is distinctive of the native membrane at a definite period of the life of the vegetative organism. For an explanation of the membrane's formative process, it is most important to examine its fine structure. Numerous studies by Frei-Vissling [5, 6], Preston [7], Wardrop [8], and other investigators who have studied the structure of cell-walls and the mechanisms of cell growth contributed much that is new to the understanding of these most complicated problems in the physiology of the vegetative cell. According to Frei-Vissling's [6] data, the growing cell membrane is a living structure; it is impregnated by cytoplasm which in situ synthesizes microfibrils. This impregnation by cytoplasm is noted at synthesis points where the cytoplasm very quickly disappears from the framework mesh of the formed membrane. At this time it is of special interest to examine the biochemical processes accompanying growth

of cell walls. These examinations were conducted on intact organs, as well as on isolated fragments consisting of growing cells.

The latter method made possible the direct study of effects of different substances on the growing cells and was successfully applied by a number of scientists, particularly by Bonner [9], Brown [10], and others for clarification of individual manifestations of the cell growth process. A biochemical study of cellulose synthesis has shown that the basic sources of synthesis are monosaccharides and chiefly their phosphate esters. In this regard, as found by Kursanov and Vyskrebentsova [11, 12], the rate of cellulose synthesis is limited by the rate of sugar supply to the point of the synthesis. According to their data, an increased supply by introduction of heteroauxin into the reaction mixture considerably increased cellulose formation as well as wall thickening of cotton plant fibers.

Investigations devoted to studies of individual biochemical processes in different root zones made possible clarification of some general features in the changing metabolism of growing tissues and, particularly, at the stage of transition of cell fission to the succeeding stage. These investigations are very complex, since they require strict cytological control, but at the same time are immensely valuable for comprehending the most detailed processes of plant life activity. Thus, in Jensen's [13] work, it was established that the cell transition from the stage of fission to the expansion stage is accompanied by essential biochemical changes. The meristematic stage is characterized by low carbohydrate content and low oxygen obsorption. The radial increase stage is accompanied by increased protein synthesis and cellulose formation, while oxygen absorption remains low. Subsequently, absorption of oxygen and water is increased and the protein content is somewhat diminished.

By using labeled glucose (glucose-1 C^{14} and glucose-6- C^{14} as substrate, Gibbs and Beevers [14] showed that in tissue differentiation the pathway of glucose decomposition is changed. In young tissues the decomposition is accomplished glycolytically, while in more differentiated tissues glycolytic decomposition is combined with oxidation.

To solve individual problems of cell growth and particularly to clarify biochemical processes of individual stages in formation of cell walls in higher plants, the cultivation of isolated tissues can also be used, and has been successfully applied in studies by a number of scientists.

White [15] was one of the first scientists widely to apply the given method in his studies and in his monograph, "Cultivation of Plant Tissues," pointed out that its application is very suitable for physiological analysis of morphogenetic factors of different cells and, particularly, those of bast fibers, stone cells, medullary rays, elements of screen-like tubes, etc.

In a series of investigations conducted on cultivations of isolated tissues, a deep interest was shown in studies on interrelations between growth substances and, particularly, between β-indol-acetic acid and individual enzymes, in the process of membrane formation [16].

Undoubtedly, such detailed biochemical studies, combined with purely chemical ones and data supplied by electron microscopy, open a channel, at this time, also for studies of formations of cell walls of more highly complex structures, which are inherent in different histological woody tissue elements of foliate varieties.

Lignification of plant tissues, related to deposites of a new component – lignin – in membranes, proceeds with a different intensity in different vegetative organisms. It is most significant in woody varieties, in which the lignin content reaches on the average 28-30% in coniferous and 20-22% in foliate plants. The appearance of lignin in cell walls, which contain phenol derivatives, is detected microscopically by different color reactions and causes fluorescence of lignified tissues under ultraviolet [2, 17, 18, 19]. Formation of woody tissues is related to the activity of a special meristic tissue–cambium [20, 21]. The character of the deposited woody tissue during the vegetative period is also different (spring and autumn woody tissue), which indicates complex biochemical processes proceeding in the cambium tissue when it functions. However, the biochemical processes proceeding in the functioning cambium and their changes when the cambium becomes a dormant so far have had little study.

The significance of woody tissue in the national economy as building material and raw material for different branches of industry is great; fields of its use are enlarged year by year. The use of woody tissue as chemical raw material is naturally related most closely to the study of composition of cell walls and the chemical changes occurring in them. This is what has evoked that large number of studies devoted to elucidating the chemical structure and biosynthetic paths of individual membrane components and particularly those of cellulose and lignin.

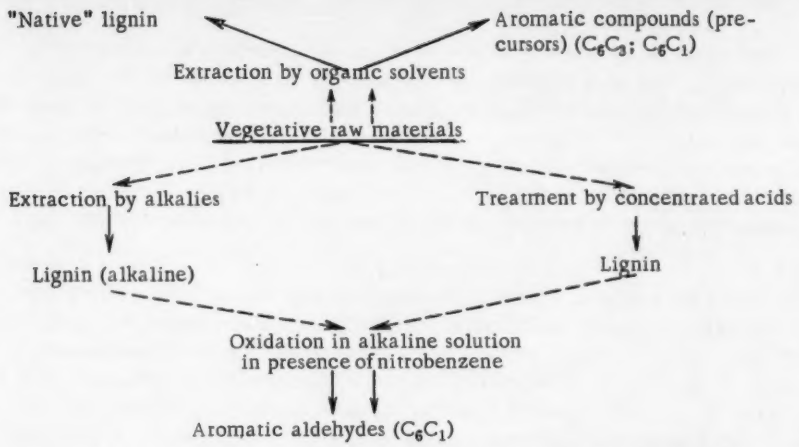


Fig. 1. An approximate scheme for isolation of lignin preparations.

The origin of the lignification process belongs to an early period in the development of plant life and results from radical qualitative changes in plant metabolism during transition to an above-ground existence. At that stage, as pointed out by Tauson [22], the appearance of and increase in lignin of membrane tissues of above-ground plants plays its part not only in increasing mechanical durability, but also serves to increase the chemical resistance of membranes with respect to effect of microorganisms.

It should be noted that lignification of cell walls occurs only during the life of protoplasts. A dead cell, devoid of cell contents, cannot be subjected to this process. And yet cells with a lignified membrane do not lose protoplasts in all cases. In such tissues as wood parenchyme and medullary rays, the lignification of cell walls does not deprive the cells of their viability. In other tissues as, for instance, tubules, the formation of a lignified membrane ends with death of the protoplast.

A study of the chemical composition of woody tissues of different ages, carried out on various items in numerous studies by Zherebov et al.[23], Sharkov et al.[24], Allsopp and Misra [25], and others, indicates that young tissues contain a large quantity of pectin and some (up to 5%) protein. An expecially large quantity of protein is contained in the forming cell walls. Thus, according to data of Thiemann and Bonner [26], oat coleoptiles contain 42% cellulose, 8% pectin, 38% polyuronides, and 12% protein. As the woody tissue ages, the pectin and uronic acid content decreases considerably and the content of cellulose and lignin is increased [2].

Interesting data were found by Sharkov and Tsverkova [27], who studied chemical composition changes of different woody varieties by the layers of woody tissue. They showed that along with composition changes in the basic woody tissue components there are also observed considerable changes in the fraction of ether-soluble substances, which denote its greater motility in the plant organism.

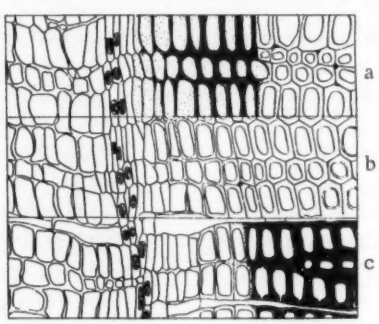


Fig. 2. Procambial woody layer a) Reaction with indicator (for β glucosidase), untreated portion; c) reaction with phloroglucinol.

The composition of the ether-soluble fraction has been little studied, although the data obtained show that this fraction may contain aromatic precursors of lignin in a free state.

According to the data of Manskaya and Bardinskaya [28], an ethereal tincture of an aqueous extract from a developing woody tissue contains aromatic aldehydes. They were found in woody tissue destroyed by fungi as well as in fossil woody tissue [29, 30].

Investigations in the last few years have shown that vanillin, lilac aldehyde (lilacin ?) and n-oxybenzaldehyde, obtained on oxidative decomposition of lignin, are found in a free state in plants where lignified tissues are found. Thus, they were found in grape seeds [31], in green and fermenting tea [32], and other plants.

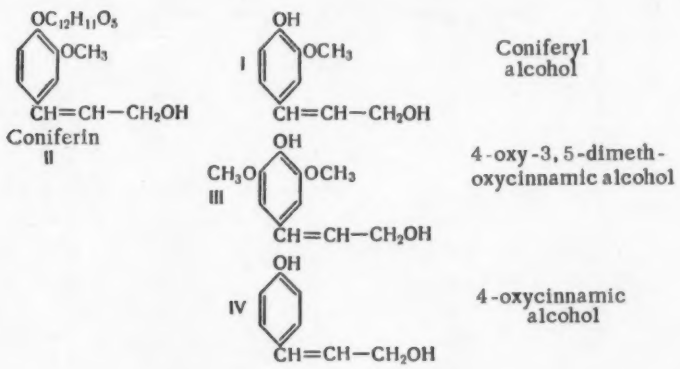
A study of the ether-soluble fraction of leaf-buds of some plants indicates the presence of small quantities of substances with a "lignin" reaction [33]. It was established that under conditions excluding oxidation the woody tissue can liberate aldehydes of a more complex structure with a three carbon side chain: coniferal and 4-oxy-3,5-dimethoxycinnamic aldehyde [34]. It should be noted that the physiological role of the aromatic lignin precursor is not clear. Together with these, such compounds as cinnamic acid or ferulic acid are among substances which have definite growth activity [35, 36, 37]. According to data of Golova, Ivanov, and Nikolaeva [38], lignin in cell membranes plays the role of an antioxidant, protecting cellulose from destruction.

With different methods of lignin isolation on the preparations obtained vary in their properties, and therefore the determination of aromatic aldehydes or other derivatives of phenylpropane in them makes possible not only comparison, but also judgment,to a certain degree,of the lignin quality in a given tissue. The possibility of different lignin qualities even in the cell wall itself is not excluded; for instance, in its central membrane or secondary layers, which is indicated by microchemical data [39], and which may be the result of different paths of lignin biosynthesis in the process of tissue differentiation, reflecting evolutionary paths in the development of a given organism. An over-all scheme for isolating lignin preparations is given in Figure 1.

The increase of lignin in cell walls is usually accompanied by an increase of methoxyl groups and aromatic derivatives. Thus, according to data obtained [28], liberation of aromatic aldehydes from lignin of the aspen precambial layer is considerably higher at the end than at the beginning of July.

A study of algal chemical composition has shown that their cell membranes contain no lignin. In moss membranes there is also no condensed lignin, like that of higher plants, but aromatic compounds, particularly aromatic aldehydes, can be found in them [40].

A chemical study of different products of lignin decomposition, model experiments with oxidative polymerization of individual possible precursors and finally, direct experiments by introducing C^{14} labeled glucosides into plant tissues, showed that the basic lignin precursor in conifera is coniferyl alcohol (I), found in plants in the form of a coniferin glucoside (II). In lignin formation in leafy varieties and grassy plants the derivatives of 4-oxy-3,5-dimethoxycinnamic alcohol (III) and n-oxycinnamic alcohol (IV) participate.



An exceptionally important place in these investigations is occupied by the studies of Freudenberg and his many collaborators [41], as well as those of Kratzl et al.[42], Hibbert [43], Shorygina [44], Nord et al.[45], and other investigators who demonstrated not only the chemical nature of lignin, but who opened paths for studying its biosynthesis in plants.

The process of forming lignin from its aromatic precursors was successfully studied by Freudenberg and his collaborators [41] and Kratzl et al [42], who studied the biosynthesis of lignin by introducing labeled glucosides-precursors into plant tissues, as well as by Siegel [46] and Higuchi [47], who investigated the process of lignin formation by introducing some aromatic compounds into tissues. Recently, interesting data were obtained by Brown and Neisch [48], who investigated possible lignin precursors by use of different compounds containing C^{14}.

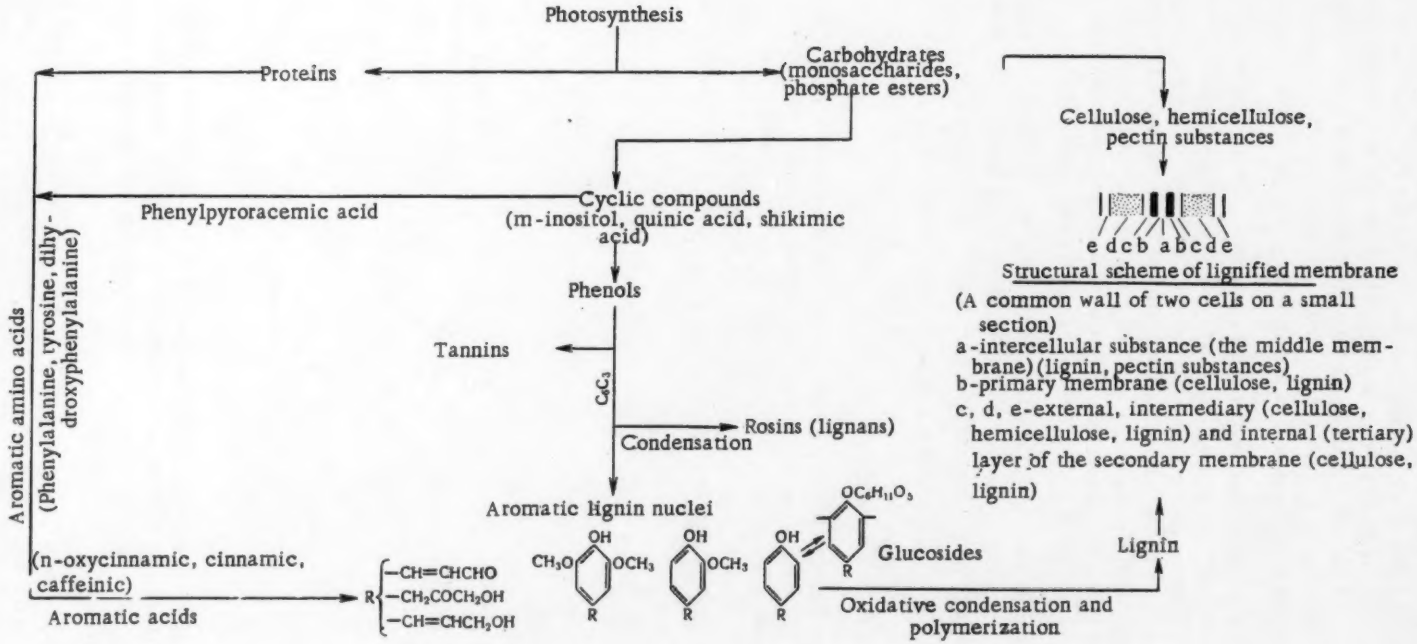


Fig. 3. Possible biosynthetic paths of cell wall components.

In studying the process of coniferin cleavage in tissues, Freudenberg also found the localization of a corresponding enzyme of β-glucosidase (Fig. 2) at spots where an intense lignification of membranes begins. Experiments on lignin biosynthesis by introducing labeled glucosides into plant tissues were subsequently confirmed by investigations of the lignification process in cultivation of tissues with the addition of corresponding glucosides — lignin precursors — into the nutrient medium. Such experiments were conducted by Wacek [49] on the carrot tissues, and also by Barnoud [50] on tissue cultures of lilac and rose.

The problem of lignin biosynthetic pathways in plants cannot yet be considered fully solved. In the studies cited above only the final stage of lignin formation was studied, i.e.,the formation of polymetic lignin from the initial aromatic precursors. The cited material indicates that the role of coniferin glucoside as a lignin precursor is basically clarified, though its physiological role in the organism is not clear. However, the enzyme systems which accomplish its biosynthesis and subsequent conversions have not yet been studied. Data on hand indicate that the group of enzymes participating in formation of lignin from aromatic precursors include β-glucosidase and oxidases, among them peroxidase. The problem of the biosynthetic paths of lignin's aromatic precursors and their physiological role in plants now becomes one of the junction points in the biochemistry of the lignification process. Repeated hypotheses,by a number of scientists,of participation by carbohydrates in the biosynthesis of lignin aromatic rings and, particularly, concepts of Klason and Vsilitsenus (see Nikitin [2]) now find experimental confirmation in studies of the biosynthesis of the phenol ring from carbohydrates via various intermediate compounds [51, 52, 53]. As investigations have shown, shikimic acid is one of the intermediates in the synthesis of aromatic compounds [54].

The study of the various aromatic compounds found in the vegetative cell indicates a close chemical relationship of aromatic lignin nuclei to other aromatic compounds which, evidently, depends upon a given community of biosynthetic paths of their aromatic precursors. Some concepts of the close relationship which exists between different aromatic substances in plants and lignin yields the scheme in the author's work [55].

Data on participation of m-inositol in the synthesis of the phenol ring, found by A. L. Kursanov et al. the close relationship of lignin to other phenol compounds of the plant cell, and finally the presence of nitrogen in lignin preparations from young plants [56, 57] allow the assumption of two possible pathways in biosynthesis of lignin aromatic nuclei from carbohydrates, and also of products of enzymatic conversions of aromatic amino acids [55, 58].

Recent studies, particularly data obtained by Brown and Neisch [48] convincingly show that the precursors of lignin can be either shikimic acid, formed from carbohydrates, which acid evidently is one of the basic links in phenol biosynthesis, or the amino acid phenylalanine and some other compounds, which confirms the possibility of several pathways in the biosynthesis of lignin aromatic nuclei.

It seems most probable that the formation of aromatic precursors of lignin proceeds along the common synthetic paths of the benzene ring from carbohydrates, through corresponding intermediate compounds. However, the possibility of participation by individual products of protoplast protein decomposition and particularly of phenylalanine in the formation of such precursors should not be excluded. This seems possible, especially in those cells where the lignification does not interfere with the normal life processes of the cell over a prolonged period, as for instance in cells of woody parenchyme or medullary rays. The hypothetically possible paths of lignin aromatic nuclei formation in the lignifying membrane are given in the diagram (Fig. 3), which is,to a certain extent,a working hypothesis in the study of biochemical paths in the individual stages of formation of a lignified cell wall.

As pointed out above, the biochemical processes which form the basis of lignification have been little studied. However, the present literature data indicate an increase to occur in oxidative processes in lignification. As was established by Joddart and Goodwin [59], formation of xylem is accompanied by an increased absorption of oxygen. In tissues of young forming woody tissue peroxidase and polyphenoloxidase are present; in addition, as found by Manskaya [60],a certain correlation between oxidase activity and coniferin accumulation is observed. The observations of increased oxidative processes in woody tissue formation permitted a number of authors [61] to hypothecate participation of substances of the coniferyl alcohol type in respiration processes of live woody tissue cells and their oxidative polymerization and condensation upon death of the protoplast. An interesting question arises: how then do the oxidative processes proceed leading to lignin formation? Do partial condensations occur of the simple, initial aromatic precursors of lignin while still in the protoplasm, or

does this process basically take place in combination of monomeric aromatic compounds with carbohydrates components of the membrane?

To clarify this problem, data are first necessary on the localization of oxidative enzymes in individual tissues and especially so in the forming membrane.

Siegel [62] obtained data indicating that the process of forming a substance similar to lignin through the effect of peroxidase on eugenol proceeds better in substrate adsorption on filter paper or in the presence of a fraction of cell walls, which indicates the significance of surface for lignin synthesis. The interesting data obtained by him need further experimental study in elucidating the role of cell-wall surface in lignification. It is important at this time to take into account the observations of Jensen [63] that there is a definite relationship between the action of β-indolylacetic acid and activity of peroxidase participating in lignification of vessels.

According to Newcomb [16], ascorbic oxidase activity of the tobacco tissue parenchyme, cultivated in vitro in partitioning by fractional centrifugation is combined with the cell-wall fraction.

The study of oxidative enzyme localization in tissues of higher plants is concerned fundamentally with their localization on the cell's structural elements [64, 65]. The enzyme localization on the protoplast surface layer has not been sufficiently studied. And yet the problem of enzyme localization on the protoplast surface layer is especially important to clarify the processes occurring in the formation of vegetative membranes.

Evidently the possibility is not excluded that the protoplast enzymes, located as they are in the surface layer and entering into direct contact with the membrane, form protein-carbohydrate complexes during certain periods of membrane formation, and this not only contributes to preservation of enzymatic activity of some enzymes for a longer period, but also to greater local activity.

The literature data indicate the possibility of formation of complexes between individual proteins, and also of proteins with enzymes and different polysaccharides. In such cases, as Rosenfel'd and Plyshevskaya [66] state in their studies, the chemical natures of the protein and polysaccharide are significant in complex formation. Separate studies indicate the possible physiological role of complex formation in the phenomena of labile enzyme protection [67, 68].

Such data indicate strongly that complex formation between enzymes and carbohydrate components of the membrane may play a definite role in the creation of given qualities in the adjacent layers of the growing cell. It should be noted that the protoplasm separation phenomenon during dormancy, as discovered by Genkel' and Oknina [69], connected with the withdrawal of protoplast from cell-walls and profound chemical reorganizations, opens paths for further study of enzymes from the bordering protoplast layer and their interaction with cell membrane components when the cell emerges from dormancy.

Summing up the data on enzymes whose participation in lignification appears probable, it is considered as established that in one of the stages of the lignification process —the formation of polymeric lignin from its aromatic precursors — β-glucosides, carbohydrases, and oxidative enzymes participate. In this regard, it is particularly important that discovery of the phenomenon of transference of carbohydrate groups to carbohydrases points to the possibilities of diversity in the formed products of the reaction in the presence of different acceptors.

The interesting data of Japanese investigators of carboligase, which can participate in the synthesis of C-C-bonds of lignin [70], are still indeterminate.

The first stage of lignification —the biosynthesis of the initial aromatic precursors of lignin — requires a study of enzymatic systems most closely related to the functioning, primarily, of the cambium and to the synthesis of phenolic compounds.

Also completely unstudied remains the final stage in the formation of lignified membrane; by what paths and through which enzymatic mechanisms does the laying down (and conversion ?) of aromatic components in the formed carbohydrate membrane proceed? Preliminary data in the literature indicate that in the study of this stage the most useful method is evidently the cultivation of plant tissues where, due to creation of definite conditions of cultivation, it is possible to observe separate moments of membrane formation as well as to clarify the interacting effect on deposition of components.

The extensive investigation of Gautheret [71] and his students conducted on cultivations of isolated tissues indicate the possibility of forming lignified vessels in such tissues under certain conditions, which opens possibilities for further biochemical studies of lignification processes.

These investigations, above all the explanation of paths for biochemical conversion of individual lignin precursors, will help to approach a study of those complex enzymatic processes which occur in formation of individual lignified tissues in the plant organism and which are conditioned by the whole history of its development.

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BRIEF COMMUNICATIONS

INVESTIGATIONS CONCERNING THE EFFECT OF DROUGHT ON SPRING BARLEY

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A great physiological problem concerns the increase of resistance of agricultural plants. Its economic significance, as well as the mystery of the phenomenon, can be considered as the reason why so many investigations aredevoted to these problems. Interesting proposals for a practical solution to this question have been suggested by Genkel' and his collaborators [1-2]. They report that a more drought-resistant plant results when, prior to planting, the seed is soaked to a moisture content of 40-45%, left in that state for two days, and then dried to its original state and planted [1-2]. This resistance applied equally to the critical period, and is hereditary.

Shkol'nik and Natanson [3] point out that a similar positive effect can be obtained when seeds are soaked before planting in a solution of boric acid, obtained by dilution of 0.5 g H_3BO_3 with subsequent drying. The data mentioned above served as a basis for carrying out investigations to test whether an analogous effect could be obtained under various conditions of climate and soil in Poland.

To obtain precisely controlled results, the experiment was set up using pot cultures, in four replicates, on podzol soil, the pH of which was 5.8, and which contained 25 mg K_2O and 8 mg P_2O_5 per 100 g of soil.

Seeds of spring barley, variety P.Z.H.R., prepared according to Genkel', as well as according to Shkol'nik and Natanson, were planted in flats. Simultaneously, unsoaked seeds were planted. The scheme of the experiment was as follows: 1) dry seeds; 2) dry seeds, drought during critical period; 3) hardened; 4) soaked in solution of microelements; 5) soaked in solution of microelements and hardened.

During the critical period, all plants (except the first treatment) were exposed to prolonged drought (18 days). The drought began during booting and ended during the beginning phase of seed formation. The intensity of the drought was significant, since the soil moisture was maintained at the level of 16% of full field capacity during the experiment. The relative humidity of the air fell to 35% during the drought. The results of the experiment are given in the table.

The data show a considerable reaction of the plants to drought (see treatments 1 and 2). When results obtained in treatments 2, 3, 4 and 5 are compared, one may note that plants in all these treatments reacted to drought in the same way. This similarity of results occurs both in the vegetative organs (straw), as well as in the reproductive ones (grain). On the basis of these results, we may propose that there was no difference in growth and development of plants in all these treatments of the experiment. Thus, the data of our experiment did not confirm the results obtained by the authors mentioned above, which, evidently, may be explained by the great complexity of the processes which cause increase in plant resistance. It may be that on soil of a different kind, or at a different moisture level of soil and air, different results might be obtained. Equally, it may be that a different reaction could have been obtained on a soil poor in microelements. One would suppose that the boron content of the soil in our experiments was sufficiently great, since plants which developed from seeds soaked in a solution of boron did not differ from the controls in their positive qualities. As was mentioned above, the investigations were carried out in a single soil, and it may be that experiments carried out in different soils would give different results.

Reaction of Spring Barley to Drought during the Critical Period, as Affected by the Methods of Preparation of Planting Material

Treatment	Harvest (in g)		Number of spikes	Plant ht (in cm)
	straw	grain		
1	21.6	17.5	22.75	75.3
2	14.9	12.4	15 75	54.3
3	14.9	12.3	14.60	54.0
4	15.6	12.8	14.60	55.0
5	15.1	12.4	16.00	53.5
	± 1.2	± 0.7	± 2.4	± 5.5

*The figures given are the average of four replicates.

In connection with the concept of the authors mentioned above, insofar as the interrelationship of plant resistance and structural and chemical characteristics of the plasma is concerned [1, 2, 3], our observations [4], as well as numerous data in the literature [6-9], evidently indicate a dependence of the resistance of plants on their energy level [4], which may affect the morphological processes. Plants which demand less energy expenditure for support of plasma structure (under unfavorable conditions) may seem to be more resistant.

The present work was carried out under the direction of Professor Dr. I. Voitsekhovskii, to whom I should like to express deep gratitude for valuable advice and cooperation.

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NUTRITION OF PLANTS WITH METHIONINE

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More and more facts have accumulated in the literature which indicate that plants need not only minerals for normal development, but also organic substances – antibiotics, amino acids, phosphoro-organic substances, etc.

Shulov [1] and Petrov [2], using the sterile culture method, have shown that higher plants can assimilate phytin and asparagine through the roots, Tanaka [3] – asparagine, glycol and cystine. Weisflog and Mengdehl – phosphorus esters of sugar. In the experiments of Hutchinson and Miller [5], pea seedlings utilized the organic compounds of nitrogen faster than the nitrates. Palladin [6] and Sabinin [7] considered that the sources of nitrogen nutrition of plants include the nitrogen of organic substances in addition to mineral salts. Krasil'nikov [8,9] established the entrance of antibiotics into plants. Kuzin and Merenova [10] have established the capacity of plants to assimilate sugars, organic acids and amino acids. Merenova and collaborators [11] obtained proofs of the direct utilization of phosphorus esters of sugars by kidney bean, barley and wheat. There are communications concerning the possibility of utilization of various organic substances by plants by Ovcharov [12], Khristeva [13], Vlassyuk[14] and Shavlovskii [15].

In our investigations on the assimilation of organic substances by plants, we used a method of aqueous nonsterile cultures, with a daily change of nutrient mixtures and with feeding of organic substances – always over a short period of time (from several minutes up to two to six hours). The root system was thoroughly washed with water. To a large degree, this prevented hydrolysis of organic substances by root secretions. Some of the experiments were carried out under sterile conditions. We borrowed the method of the sterile experiments from Fedorov [16] and Shulov [1].

The amino acid methionine used in the experiments was labelled with S^{35}. The experimental plants were grown from seeds. Feeding of plants was carried out in water cultures, the activity of the solutions varying from 4 to 7 μ C/ml.

TABLE 1

Assimilation of Radioactive Sulfur by Mustard Leaves after Methionine Feeding (counts/minute per 100 mg)

Leaves	Duration of feeding				
	15 min	30 min	1 hr	2 hr	24 hr
Seventh	8	182	488	3113	15,493
Fifth	0	31	45	131	3,136
Third	–	–	–	69	500

TABLE 2

Weight (in g) of Living Plants and Seeds Fed Methionine and Sodium Sulfate (24 plants)

Plant	Nutrition	Weight of aerial mass and roots				Weight of Seeds
		April 25	May 28	July 1	August 19	
Rice	Methionine	3.8	44	152	206	8.0
Mustard	The same	3.7	38	38	Harvested	1.7
Rice	Sodium sulfate	4.1	31	117	152	2.0
Mustard	The same	3.1	38	34	Harvested	1.2

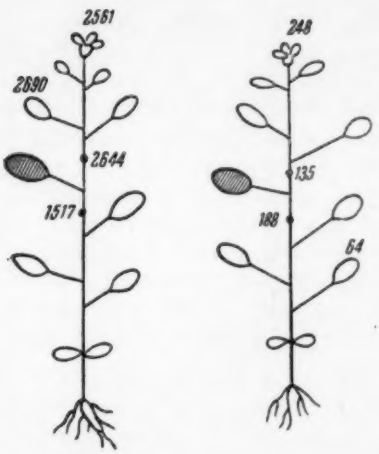

Fig. 1. Experimental scheme of foliar feeding of mustard, variety Sareptskaya with methionine and sodium sulfate. Application to the leaf for a period of 6 hours. Activity measured after 13 hours of translocation. Figures are given in counts/minute per 100 mg tissue. Leaf fed is shaded. At the left) feeding with methionine; on the right) with sodium sulfate.

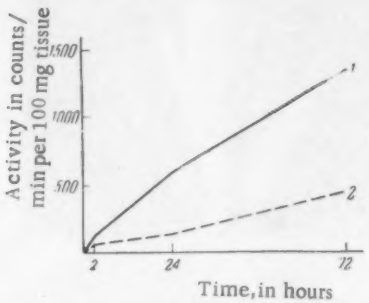

Fig. 2. Accumulation of radioactive sulfur (S^{35}) in the spike of wheat, Al'-bidum 43, when fed methionine and sodium sulfate. 1) Fed methionine; 2) fed sodium sulfate.

Fig. 3. Radioautograph of corn. At the left) S^{35} given as methionine, at the right) as sodium sulfate.

Experiments carried out in 1953-1954 [17] have shown that, during root feeding of mustard, the methionine passes rapidly through the root system into leaves and other organs. After root feeding with methionine, the radioactive sulfur went mainly into young leaves, in which growth processes were most active (Table 1).

The greater accumulation in actively growing organs of the plant of sulfur from methionine, as compared with sulfur from sodium sulfate, was established in experiments with foliar feeding of mustard during flower-bud formation (Fig. 1).

Fig. 4. Radioautograph of corn. Root feeding of plants with methionine under sterile conditions.

Similar results have also been obtained with root feeding of methionine and sodium sulfate to spring wheat. Plants of spring wheat, Al'bidum 43, in the beginning of spiking were dug out of the field, washed, and placed in vessels of water. One group of plants was given methionine, the other sodium sulfate. The radioactive sulfur (S^{35}) activity of both solutions was strictly identical, 7 μC/ml. Two hours after the beginning of feeding, the plant roots were washed and placed in tap water. For measurement of radioactivity, samples of plants were taken 2, 24 and 72 hours after the beginning of feeding. The experiment was repeated 8 to 10 times. The results of activity measurements in the spike are given in Fig. 2.

Comparative assimilation of sulfur from methionine and sodium sulfate in corn was established by a radioautographic technique. In this experiment, corn, variety Spasovskaya, was grown in the usual nutrient medium, and then transferred to radioactive solutions of methionine and sodium sulfate. The S^{35} activity in each solution was 4 μC/ml. Five and one-half hours after feeding, the aerial mass of the plants was cut, dried and placed on photographic film (Fig. 3). For a control, the experiments in feeding methionine (S^{35}) to corn, variety Spasovskaya, were carried out under sterile conditions. The radioautographs obtained (Fig. 4) show a considerable content of sulfur from methionine in the young leaves.

To make certain not only of the intake, but also of the assimilation of organic compounds, experiments were set up with prolonged cultivation of plants (until fruit-bearing) under nonsterile culture conditions. As experimental plants we used rice, variety Dubovskii, and mustard, variety Sareptskaya. Organic sulfur was supplied as methionine, inorganic sulfur, as sodium sulfate. Plants received these compounds for five hours every other day. The remainder of the time, the plants were placed in nutrient mineral media containing no sulfur. Calcium and magnesium sulfates, included in the usual media (Hellriegel, Pryanishnikov), were replaced by chlorides of the same elements. S^{35} activity was 0.8 μC per liter in both the methionine and sodium sulfate solutions. The total sulfur and nitrogen content was also the same in both solutions.

In 87 days, plants of rice and mustard which received methionine accumulated considerably more organic matter, and formed more seeds than plants which were given sodium sulfate (Table 2).

Leaves and seeds of plants fed methionine contained considerably more radioactive sulfur than those fed with sodium sulfate. Thus, for example, the activity of mustard seeds was respectively 15.2 and 4.3 thousand counts/minute per 10 mg.

Analysis has also shown that more methionine sulfur than sodium sulfate was accumulated in the proteins (globulins) extracted from seeds. The activity in rice was 18.5 thousand counts/minute per 100 mg protein and 6.1 thousand, respectively, and in mustard was 45.2 thousand and 17.7 thousand.

Better development of plants, as well as greater activity in their leaves, seeds and proteins, indicate the introduction of methionine sulfur into the plant without a previous breakdown, and its participation in the synthesis of organic substances.

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CONCERNING SOME METABOLIC PROCESSES IN THE ASSIMILATION OF SULFUR BY PLANTS

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Sulfur is utilized by plants exclusively in the oxidized form (SO_4), i.e., as salts of sulfuric acid [1, 2]. Numerous attempts to carry out nutrition of plants with the reduced form of sulfur, in particular in the form of thiourea, have not as yet given positive results. A considerable part of the sulfur introduced into the plants in the oxidized form is later reduced, and in the reduced form enters into many organic substances and accomplishes in plants the most important physiological functions [1, 2].

The process of sulfur reduction is inevitably connected with the oxidation of other organic substances [3-5]. Organic compounds which are first subjected to oxidation include sugars. This gave a basis for the proposal that the process of sulfur assimilation by plants is connected with a change in carbon metabolism [7].

For an experimental test of this proposal, in 1956-1957, in the Plant Nutrition Laboratory of the All-Union Institute of Fertilizers and Agricultural Soil Science (VIUA), we carried out several vegetative experiments with plants characterized by varying contents of sugars.

In the experiments of 1956, we chose hemp (variety YuS-1) as a plant containing very few sugars in the young stages and corn (variety Voronezhskaya 76) as a plant rich in sugars. In 1957, monoecious hemp and corn ("Pionerka Severa") were used for the experiment.

Plants were grown in containers holding 8 kg of soil. The soil was a medium-carbonaceous peat-podzol, with a pH of 4.8 in its salt extract. In 1957, this soil was treated with lime, and its pH brought to 6.8. As a common background, 1.5 g P_2O_5, 1.5 g K_2O and 1 g of nitrogen were applied to every container in the course of the growing period, in four applications. In treatment 4, an amount of calcium equivalent to calcium nitrate was applied in the form of $CaCO_3$ (chalk). At the time the containers were filled, the fertilizers were applied as a solution which was mixed with the soil, while during growth of the plants, the fertilizers were placed on the soil surface.

Samples of plants in all treatments were taken simultaneously, and only on sunny days, from 9 to 10 A.M. These samples were killed at a temperature of 100° for 5 minutes, and were dried to an air-dry state of 60°. The samples were then ground and sifted through a sieve of 1 mm mesh. The dry material was extracted with water, and the extracts were analyzed for sugars and for soluble and protein nitrogen. Sugars were determined by Bertrand's method, and nitrogen by the Kjeldahl method. Determinations of free amino acids were carried out by monodimensional chromatography.

As distinct from corn, sunflower, pumpkin, and other crops, hemp secretes a very insignificant amount of cell sap, and there is no practical way in which hemp cell sap can be analyzed. Analysis of leaves, where the synthesis of proteins from amino acids predominantly takes place, is also inapplicable. Because of this, we used the lower part (1/3) of hemp stems in our investigations. The amino acid content in this part of the stem may, to a certain degree, characterize the composition of the amino acids which enter into the roots and aerial organs. For this purpose, fresh material was fixed with hot 96% ethyl alcohol for 20 minutes. The amino acids

TABLE 1

Changes in Sugar Content during Assimilation of Sulfur by Plants

Treatment No.	Form of fertilizer	Sugar, as % of dry weight			Sugar content, mg per 100 plants			
		reducing	sucrose	soluble	reducing	sucrose	sum	in %
I. Hemp - planted May 22, analysis of aerial mass July 28, 1956								
1	NH_4OH	2.11	1.58	3.69	2701	2022	4723	100
2	$(NH_4)_2SO_4$	2.08	0.10	2.18	1623	69	1692	36
3	NH_4OH + Ca equivalent	5.10	0.78	5.78	11100	1731	12831	250
4	$Ca(NO_3)_2$	0.80	1.54	2.34	704	1355	2058	43
II. Hemp - planted June 13, analysis of aerial mass July 4, 1956								
1	NH_4OH	5,60	3.23	8.83	1736	1001	2737	100
2	$(NH_4)_2SO_4$	1,39	1.95	3.37	170	235	404	15
3	NH_4OH + Ca equivalent	4,07	3.37	7.24	1120	1045	2165	80
4	$Ca(NO_3)_2$	1,34	0.62	2.21	207	81	288	10
III. Hemp - planted May 16, analysis of third or fourth leaf, June 21, 1957								
1	NH_4OH	3,07	2,13	5,20	Determination not made			
2	$(NH_4)_2SO_4$	1,52	2,08	3,58	Ditto			
3	NH_4OH + Ca equivalent	2,25	2,56	4,81				
4	$Ca(NO_3)_2$	1,50	1,63	3,13				
IV. Hemp - planted May 22, analysis of aerial mass June 12, 1956								
2	$(NH_4)_2SO_4$	5,15	1,34	6,49	1287	335	1629	50
3	NH_4OH + Ca equivalent	8,98	0,96	9,94	2963	317	3280	100
4	$Ca(NO_3)_2$	3,86	2,50	6,36	1119	725	1844	56

TABLE 2

Nitrogen Content in Plants Assimilating Sulfur (SO_4) and Nitrate Nitrogen (NO_3)

Treatment no.	Fertilizer form	Nitrogen, % of dry weight			Nitrogen, mg per 100 plants			
		soluble	protein	total	soluble	protein	total	in %
I. Hemp - planted May 22, analysis of aerial mass July 23, 1956								
1	NH_4OH	0.43	3.77	4.22	550	4826	5576	100
2	$(NH_4)_2SO_4$	0.43	4.16	4.59	331	3203	3534	65
3	NH_4OH + Ca equivalent	0.43	3.12	3.55	1155	6726	7144	133
4	$Ca(NO_3)_2$	0.54	4.17	4.71	493	3670	4163	77
II. Hemp - planted June 13, aerial mass analyzed July 4, 1956								
1	NH_4OH	0.42	3.09	3.52	130	958	1088	100
2	$(NH_4)_2SO_4$	0.55	3.52	4.07	70	422	492	45
3	NH_4OH + Ca equivalent	0.45	3.20	3.65	129	1022	1151	106
4	$Ca(NO_3)_2$	0.50	3.30	3.80	65	429	494	45

were then extracted by six portions of 75% alcohol. The alcohol extract was evaporated on a water bath. The residue was dissolved in water, filtered, again evaporated to dryness, and the amino acids were dissolved in 1 ml of water, acidified with hydrochloric acid.

TABLE 3

Effect of the Form of Nitrogen Fertilizers as Ammonia, Nitrates, and Sulfur on Content of Free Amino Acids in Hemp Stems (planting May 16, analysis July 26, 1957; determinations by monodimensional chromatography)

Treatment No.	Amino acid	Amino acid content, mg per 100 g wet weight		
		NH_4OH	$(NH_4)_2SO_4$	$Ca(NO_3)_2$
1	Cystine + cysteine	Absent	Traces	Traces
2	Ornithine	Traces	The same	The same
3	Lysine	The same	4.1	4.1
4	Histidine	Absent	Traces	Present
5	Asparagine	The same	The same	4.9
6	Arginine	" "	" "	Traces
7	Aspartic acid	2.0	8.1	5.3
8	Serine	0.3	0.5	0.6
9	Glycine	Absent	Absent	Absent
10	Glutamic acid	1.6	3.3	3.6
11	X-substance	Traces	Traces	Present
12	Alanine (α + β)	2.3	3.3	2.6
13	Proline	Absent	Present	Much
14	Tyrosine	2.1	Traces	Absent
15	Tryptophane	Absent	Absent	5.3
16	Valine + phenylalanine	4.3	13.5	17.6
17	Leucine + norleucine	2.1	3.3	6.0
18	Total amino acids	14.7	36.1	50.4

Quantitative determinations of amino acids were carried out by monodimensional paper chromatography, using n-butanol, acetic acid, water in a ratio of 4:1:1 by volume. With a micropipette, the amino acids were placed on the paper in amounts of 0.0171-0.0285 ml.

The amino acids were separated by running the solvent through the paper five times, with exposures of 5, 8, 13, 19 and 29 hours (for separation of amino acids with an R_f greater than 0.4, located in the lower part of the chromatogram – alanine, proline, tyrosine, tryptophane, amylobutyric acid, valine, phenylalanine, leucine) and with exposures of 5, 8, 19, 24 and 105 hours (for separation of the amino acids located in the upper part of the chromatogram – cystine, ornithine, lysine, histidine, asparagine, glutamine, aspartic and glutamic acids, serine, leucine and threonine).

Identification of amino acids was made by means of standard solutions, and by development of chromatograms with a 0.5% solution of ninhydrin in acetone with subsequent exposure for 36 hours in the dark, at room temperature.

Different amino acids gave different colors with ninhydrin – varying from pink to intense violet, depending on the nature and amount of the amino acid. This caused considerable difficulty in quantitative determination of amino acids in the colorimeter. Because of this, following the ninhydrin treatment, the chromatograms were treated with a solution of cupric nitrate in acetone (1 ml of a saturated solution of $Cu(NO_3)_2$ in 100 ml acetone). With this solution, all amino acids gave a consistent red color.

The colored spots of amino acids were cut out, and the stain was extracted with 6 ml of methyl alcohol for several hours. The optical density of the solution was measured against a control on an FEK-M photoelectric colorimeter with a blue filter (λ = 420 μ), in cuvettes of 10 ml volume. The amino acid content was calculated from the respective calibration tables.

For construction of the calibration curves, standard solutions of each amino acid were chromatographed under the same conditions as the experimental solutions, and were prepared for photocolorimetric measurement in exactly the same way. The data presented are averages of four parallel determinations.

In Table 1 are given the changes in the content of sugars in leaves and aerial mass of hemp and corn under the influence of the assimilation of sulfur and of nitrogen in the form of nitrate.

Comparison of the sugar content in the aerial mass of hemp and corn at a young age shows that the amounts of sugars are considerably smaller when sulfur is supplied as ammonium sulfate than when plants are given aqueous solutions of ammonia.

An analogous phenomenon is observed on comparing plants which received nitrogen in the nitrate form as well as in the form of aqueous solutions of ammonia (treatments 3 and 4). Here, one should note that the decrease in sugars in young plants which assimilate oxidated sulfur and nitrate nitrogen is not accompanied by an intensification of protein synthesis (Table 2).

While no significant difference was found in the total protein content when plants were given ammonia, nitrates and ammonium sulfate, a significant difference in the content of free amino acids was found in hemp stems (Table 3). With sulfates and nitrates, as compared with ammonia, the content of lysine, aspartic, and glutamic acids, valine, phenylalanine, leucine, norleucine, and the total content of free amino acids are considerably increased.

Along with these differences, in some processes the assimilation of nitrates and sulfur by plants have similar effects, as is shown by the free amino acids. This gives us a basis for considering that the decrease of sugars in plants during assimilation of sulfur occurs as a result of their expenditure (oxidation) in the reduction of the oxidized sulfur to the reduced form. This process, in its biological nature, is very close to the process of reduction of nitrates (NO_3) to ammonium (NH_4). As a consequence of this, in some processes the sulfur compensates for a deficiency of nitrates, and the latter may compensate for sulfur. Because of this, when plants are abundantly supplied withnitrates, the reduction of SO_4 is retarded, and vice versa.

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EFFECT OF FOLIAR APPLICATION OF THE MICROELEMENTS MOLYBDENUM AND NICKEL ON SOME METABOLIC PROCESSES IN THE POTATO PLANT

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In the agricultural practice, the application of microelements is used more and more to increase productivity of animal and plant organisms. At present, there is a great deal of literature on the effect of macro- and microelements on various aspects of the vital activity of plants, when applied to leaves [1 and others]. It should be noted that the greater part of the experimental data on foliar feeding of plants with microelements is devoted to the study of its effect on size and quality of yield. However, the successful application of foliar feeding with microelements is impossible without a detailed knowledge of their physiological action on the plant organism under the specific conditions of its growth.

Lately, a number of investigators have clearly proven the necessity of the microelement molybdenum for increase in yield and quality of production of beans and cauliflower [2,3]. This effect is related to the fact that molybdenum is a part of the nitrate-reductase enzyme and has a great effect on nitrogen and carbon metabolism. There are a small number of papers concerning the effect of molybdenum on various living processes of plants, but these touch on only a narrow range of problems. We know of one paper by Dobrolyubskii [4], in whose experiments root and foliar feeding of grape with nickel sulfate led to more rapid ripening and to better fruit quality.

The purpose of the present work is the study of the physiological-biochemical shifts caused by foliar feeding of potato plants with solutions of molybdenum and nickel salts, and the establishment of the relationship between the physiological indicators and yield.

The investigation was carried out with potato, variety Stakhanovskii, which was grown on the degraded chernozem soil of the experimental station of the Ukrainian Research Institute of Genetics and Selection (Khar'kov).

For microfertilizers, we used 0.0001 M solutions of nickel sulfate and sodium molybdate, with whcih plants were sprayed four times during growth, every other week. Control plants were sprayed with distilled water.

In our physiological-biochemical investigations, we limited ourselves to determination of the content of nucleic acids (RNA and DNA), nitrogenous substances and carbohydrates in leaves. In addition, we determined the activity of catalase, the content of reduced glutathione, total hydration, the value of the saturation deficit of separate leaves, and the amount of "wet" ash in leaves.

Isolation of nucleic acids from the dry matter of leaves was carried out by the modified method of Schmidt and Tanhauser [5]. Nucleic acidphosphorus was determined by the Fiske-Subbarow method using an FÉK-M photocolorimeter. Reduced glutathione in leaves was determined by the modified method of Woodworth and Free [6]. Extraction and determination of nitrogen in protein substances was carried out by the method described by Kursanov [7]. Sugars were determined by Bertrand's method, as modified by Lisitsin [8]. The amount of

TABLE 1

Effect of Molybdenum and Nickel on Water and "Wet" Ash Content of Potato Leaves

Treatment	Water, %	g ash per g dry matter	g dry matter per g ash
Before flowering			
Water – control	80.80	0.0783	12.77
Water + Mo	80.89	0.0942	10.61
Water + Ni	81.03	0.0927	10.89
After flowering			
Water – control	79.91	0.0813	12.30
Water + Mo	80.37	0.0865	11.66
Water + Ni	80.24	0.0825	12.12

starch was calculated as the difference between the sum of carbohydrates after 3-hour hydrolysis with 2% HCl and the sum of water-soluble carbohydrates after 5-minute hydrolysis with 2% HCl. Catalase was determined gasometrically with the apparatus described by Kalinin and Yastrembovich [9] Throughout the whole growing period, young leaves – the second from the tips – were analyzed. At least two parallel determinations were carried out in each analysis, giving a close correspondence in results. Our investigations concerning each indicator include not less than 8 to 10 consecutive experiments in the course of the growing season, the results of which, as a rule, were in the same direction. Taking this as a basis, the data of our experiments on most indicators are summarized according to two growing periods, before flowering and after flowering, and the numerical figures of the tables represent average data of four to five experiments, carried out in every growing period.

TABLE 2

Effect of Molybdenum and Nickel on Content of Phosphorous Compounds in Potato Leaves (P_2O_5) in mg per g dry matter; average of 5 consecutive experiments in the course of a month)

Treatment	Total P_2O_5	Phosphatides	In % of total	Mineral P_2O_5	In % of total
Water – control	18.48	3.18	17.2	7.87	42.5
Water + Mo	17.60	3.42	19.4	6.35	36.0
Water + Ni	18.28	3.36	18.3	6.30	35.0

Microelements which enter leaf tissue through foliar application undoubtedly must have an effect on the physicochemical state of protoplasm.

One of the indicators which characterize the state of protoplasm colloids is the water regime of leaf tissues, which is related to the properties of enzymes and to other characteristics of metabolism [10].

In Table 1 are given data on the effect of spraying plants with molybdenum and nickel salts on the total hydration of upper leaves and their "wet" ash content.

It follows from the data of Table 1 that under the influence of molybdenum and nickel the upper leaves, as a rule, contain more water than those of control plants.

It should be mentioned that Shkol'nik [11] also observed an increase in water content in leaves of wilting wheat, under the influence of the microelements boron, copper, manganese and zinc. In leaves which received supplementary feeding with solutions of molybdenum and nickel salts, the amount of ash was increased, which probably indicates a more intensive assimilation of mineral substances from the soil.

Determination of the saturation deficit in leaves cut under water at 10 A.M. on hot days (air temperature from 25 to 29°) has shown that plants which receive microelements absorb 1-2% less water than control plants, which may be explained by a higher total hydration of the tissues of experimental plants.

It is possible that molybdenum and nickel lower the rate of transpiration of water through the leaves. In our experiments, transpiration was not specifically studied, but it is known from data in the literature (Abutalybov [12]) that transpiration is decreased in the wilting leaves of wheat under the influence of copper, zinc and manganese.

TABLE 3

Effect of Molybdenum and Nickel on Nucleic Acid Content of Potato Leaves (P_2O_5 in mg per g dry matter; average of five experiments)

Treatment	Total nucleic acids	RNA	DNA	DNA/RNA	Treatment	Total nucleic acids	RNA	DNA	DNA/RNA
	Before flowering					After flowering			
Water - control	5.08	4.08	1.00	0.24	Water - control	3.76	3 00	0.76	0.25
Water + Mo	5.32	4.03	1.29	0.32	Water + Mo	3.87	2 86	1.01	0.35
Water + Ni	5.33	3.84	1 48	0.38	Water + Ni	3.87	2.95	0.92	0.31

TABLE 4

Effect of Molybdenum and Nickel on Carbohydrate Content of Potato Leaves

Treatment	mg per g dry matter			starch in % of total
	sum of water-soluble	total sum of strong hydrolysis	starch	
Before flowering (average of 5 experiments)				
Water - control	33.70	146.2	111.50	76.9
Water + Mo	31.90	153.6	121.70	79.2
Water + Ni	26.15	134.8	108.67	80.6
After flowering (average of 4 experiments)				
Water - control	48.50	122.50	74.0	60.4
Water + Mo	45.20	120.00	74.8	62.3
Water + Ni	39.5	112.50	73.0	64.8

TABLE 5

Effect of Molybdenum and Nickel on Content of Nitrogenous Substances in Potato Leaves (in mg per g dry matter)

Treatment	total extractable with 0.3%	Protein (a)	Nonprotein (b)	Ratio a/b
Before flowering				
Water - control	10.18	5.22	4.96	1.05
Water + Mo	12.81	7.47	5.35	1.40
Water + Ni	9.68	5.99	3.69	1.65
After flowering				
Water - control	10.80	4.38	6.42	0.68
Water + Mo	10.34	5.05	5.30	0.95
Water + Ni	11.06	5.62	5.44	1.03

In Table 2 are given data on the study of the effects of molybdenum and nickel on the transformation of phosphorous compoundsin leaves (determinations carried out according to A. V. Sokolov's method). From Table 2, it can be seen that when microelements are given through the leaves, there is a decrease in the total amount of P_2O_5, as well as its mineral form, which is the most mobile and is the starting point for all other forms of phosphorous compounds on the other hand, the content of phosphatide phosphorus is considerably increased, which also indicates the activation of the synthetic activity of the leaf.

The data of Table 3 show that the total nucleic acid phosphorus content rises under the influence of molybdenum and nickel and that there is an increase in the ratio of DNA to RNA phosphorus, i.e., the ratio of nuclear nucleic acid to cytoplasmic nucleic acid, which, evidently, is conditioned by the fact that the microelements, which increase the hydration of the upper leaves (see Table 1), lead to an increase in the number of rapidly-dividing cells with a higher nucleoplasmic ratio than in control plants. However, the possibility is not excluded that microelements may also have a direct action on the synthesis and transformation of nucleic acids. It is possible that molybdenum and nickel, through oxidation-reduction reactions, contribute to a decrease in the process of oxidation and depolymerization of DNA.

On analyzing the data of Table 4, it can be seen that under the action of molybdenum and, particularly, of nickel, the amount of water-soluble carbohydrates is decreased, and the starch content, shown as the sum of carbohydrates of strong hydrolysis, is somewhat increased.

Table 5 gives the data of analyses of the effect of molybdenum and nickel on the content of alkali-soluble nitrogenous substances in potato leaves.

From the data of Table 5, one may conclude that foliar feeding of potato with molybdenum and nickel causes an increase in the ratio of protein to nonprotein nitrogen, and this indicates that molybdenum and nickel activate the synthesis of alkali-soluble protein substances in the upper leaves of potato.

Determination of catalase activity and glutathione content in leaves has shown that the same concentration of molybdenum and nickel is optimal for activation of catalase and is somewhat inhibitory for SH-glutathione. In generalizing the data we obtained, we may conclude that foliar supplementary feeding of potato with molybdenum and nickel improves the water regime of leaves and decreases their water deficit during hot days, as a result of which there occurs an activation of metabolism, which is expressed in acceleration of the synthetic activity of upper leaves.

On this basis, one can explain the increase in yield which was observed in our experiments. Spraying plants with molybdenum increased the yield of tubers per plant by 14%, as compared with controls, while spraying with nickel caused an increase of 12% per plant.

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EFFECT OF FOLIAR NUTRITION AND SOIL MOISTURE ON THE GROWTH AND DEVELOPMENT OF MAIZE

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From data in the literature [1-6, and others], as well as from agricultural practice, it is known that application of various mineral fertilizers to the soil may shorten or prolong the duration of the vegetative period of plants, increase their productivity, and improve the quality of the yield.

Numerous experiments of a number of investigators have shown a favorable effect not only of nutrition through the roots, but also of foliar application of mineral fertilizers; this was summarized in the special monograph of F. F. Matskov on the foliar nutrition of plants [7], as well as in a collection of translations from the foreign periodical literature on this question [8]. However, it can be seen from F. F. Matskov's critical review of the large number of papers on foliar nutrition of plants that, in different experiments, the same mineral fertilizers have far from similar physiological actions on the growth and development of plants. This is also supported by the latest studies of a number of authors, and depends on the form of nutrients [7-11], concentration of solutions [8,9], soil and climate conditions [12-14], time and amount of applications [7,15], and also on specific plant properties [12].

Insofar as the effect of soil moisture on the efficiency of foliar application of mineral fertilizers is concerned, we know of only a few papers on the subject, and these are quite contradictory in nature. Thus, for example, in the experiments of Krasinskii [16], carried out in the Saratovskaya region, foliar nutrition of the same crops gave more favorable results under the conditions of the hot and dry summer of 1955 than in 1956, when there was much precipitation, with a temperature relatively similar to that of 1955.

Taking the above into consideration, we chose, in one of the vegetative experiments of 1957, to follow the effect of foliar application of mineral fertilizers on certain physiological indicators and on productivity of maize under various soil moisture conditions. The work was carried out in the water regime laboratory of the K. A. Timiryazev Institute of Plant Physiology, USSR Academy of Sciences.

METHODS

The experiment of 1957 was carried out in containers with a capacity of 7 kg of soil. The soil used for filling the containers was 50% peat-podzol, 35% humus, and 15% quartz sand. Throughout the whole growing period, the plants were grown under two sets of conditions: one series of experiments was carried out at a soil moisture of 35% of full-field capacity; the second, at 70% of full-field capacity. The foliar application was made three times during the growing period: first, during appearance of the eighth leaf; second, in the beginning of tasselling; third, during anthesis. The following fertilizers were used for foliar feeding: nitrogen, as NH_4NO_3; phosphorus, as superphosphate; potassium, as KCl. The concentration of the solution at the first application was 3% (30 g fertilizer per liter of water), but since burns appeared on the leaves at this concentration, the second feeding was made with 2%, and the third feeding with 1% solutions, with the exception of the NPK treatment, in which the solution concentration was kept at 3%.

The experiment was carried out in six replicates. Five seeds were planted in each container, but after the fifth leaf appeared, the plants were thinned to leave the three most similar plants. Later, plants were cut down

Some Physiological Indicators of Growth and Development of Maize, Variety R-3, during Foliar Feeding with Mineral Fertilizers (average data for the period from June 10 to August 2, 1957, calculated per container from 18 determinations)

Treatment No.	Treatment	Amount water used, kg	Accum. of absolutely dry mass, g	Transpiration rate, g/kg	Total leaf surface, in^2	Rate of photosynthesis, g/in^2
1	Total leaf surface, in^2	8.54	26.2	3.0	13.4	2.0
2	The same, 70% soil moisture	11.22	44.5	4.0	26.1	1.7
	Foliar feeding, 70% soil moisture					
3	Nitrogen (N)	13.24	36.1	2.7	26.1	1.6
4	Phosphorus (P)	11.92	47.4	3.9	22.9	2.1
5	Potassium (K)	9.84	44.5	4.5	26.8	1.6
6	NPK	11.10	59.6	5.1	28.4	2.1
7	NPK, 35% soil moisture	6.04	15.8	2.5	8.6	1.8

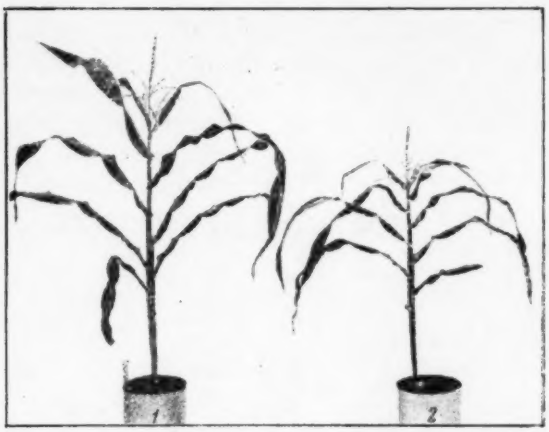

Fig. 1. Effect of foliar feeding with NPK on growth and development of maize at 35% soil moisture. On the left – control; on the right – fed NPK

for analysis, and toward harvest time only one plant was left per container; however, in any given growing period, the number of plants was the same in all containers. Seeds were planted in the containers May 17, and the plants were harvested September 12. The maize variety used was "Stednespelyi R-3".

To determine the extent of the effect of foliar feeding of maize at different soil moisture values on the growth and development of plants, we measured the utilization of water by the plants throughout the whole growing period, by daily watering by weight. Periodically, we determined the water regime of the plant amount of total, free and bound water), the osmotic pressure of cell sap, the increase in total leaf surface, the accumulation of organic matter, and also the rates of transpiration and photosynthesis. Part of the data obtained is presented below.

EXPERIMENTAL RESULTS

In all experimental treatments, foliar feeding at 35% soil moisture gave negative results, as can be seen from the Table.

It can be seen from the Table that maize plants use water most economically during foliar feeding with NPK, on a background of 70% soil moisture. With deficient moisture, the same fertilizers greatly decrease the transpiration efficiency. An analogous situation was observed during foliar feeding with nitrogen alone, when this element was present in excess in the soil (considering the addition of 35% humus to the soil in filling the containers).

Insofar as the accumulation of organic matter, size of leaf surface and efficiency of photosynthesis are concerned, the same relationship exists as with transpiration efficiency. In the case of photosynthetic efficiency, the control at 35% soil moisture is an exception. But this is explained by the fact that part of the lower leaves in this treatment dried out prematurely, and the accumulated organic mass is actually related to a smaller leaf surface, which does not reflect the actual situation with respect to photosynthetic efficiency in this treatment.

Fig. 2. Effect of foliar feeding with NPK on growth and development of maize plants at 70% soil moisture. On the left – control; on the right – fed NPK

Plants in all experimental treatments were photographed on July 30. Two of the most characteristic photographs are presented in Figures 1 and 2.

Thus, our vegetative experiments with maize did not confirm the data of Krasinskii [16] concerning the positive effect of foliar feeding of plants during drought. In the experiments of Shereverya [17], during a low water supply level, foliar feeding suppressed the root feeding of spring wheat, and at the same time decreased its yield, which is completely in accord with the results of our experiments.

It is known that the root system of plants has an important role in the synthesis of organic matter in aerial organs. In this connection, we attempted to determine the effect of foliar feeding of maize at different soil moistures on the activity of the root system. To a certain degree, this may be determined by observing plant guttation and by analysis of the guttation fluid by the method of paper chromatography. The qualitative composition of the guttation fluid in different experimental treatments can be seen on the chromatograms (Fig. 3).

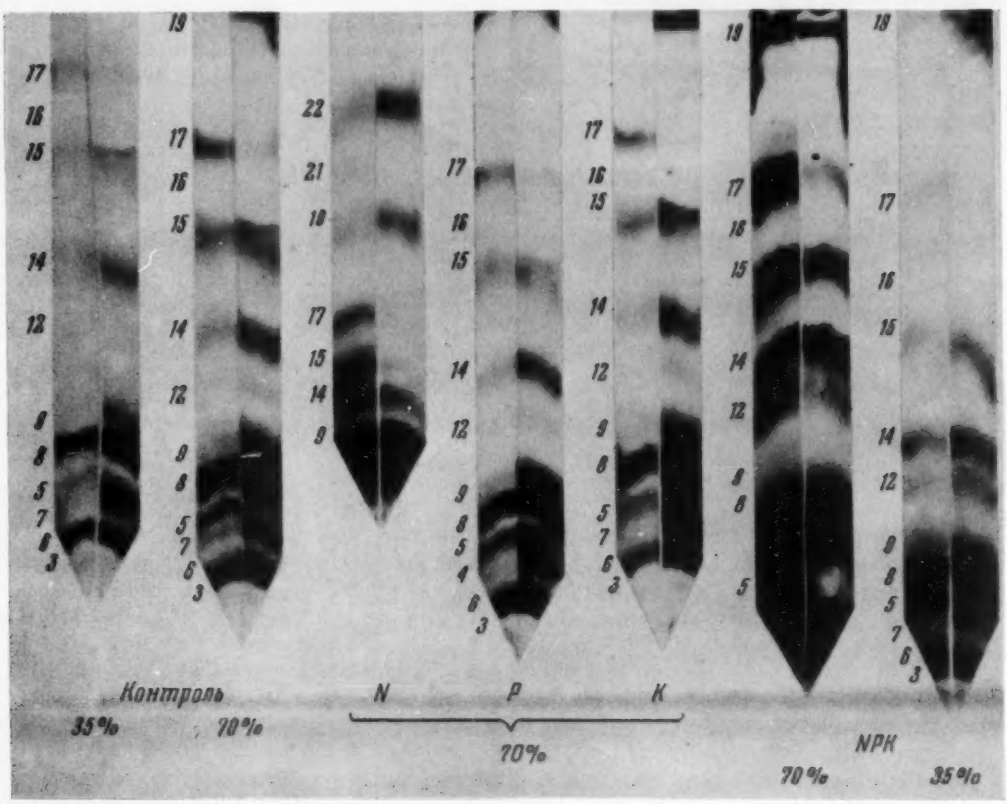

Fig. 3. Effect of foliar feeding of maize by mineral fertilizers on synthesis of amino acids at different soil moistures. 3) lysine; 5) asparagine; 6) histidine; 7) arginine; 8) aspartic acid; 9) glutamine; 12) glutamic acid; 14) threonine; 15) alanine; 16) proline; 17) tyrosine; 19) valine; 21) phenylalanine; 22) isoleucine.

It can be seen from the chromatograms that the most intensive synthesis of amino acids occurs in the root system of maize plants fed NPK through the leaves and grown at 70% soil moisture, which had a very favorable effect on the growth and development of plants (see Fig. 2, plant on the right). The smallest concentration of amino acids occurred in the guttation fluid of plants grown with insufficient soil moisture. In plants fed with a single one of the mineral fertilizers – nitrogen, phosphorus or potassium – at 70% soil moisture, feeding with potassium somewhat increases the amino acid concentration in the guttation fluid as compared with phosphorus and, particularly, with nitrogen. In the latter case, evidently, the inhibition of plants takes place from the excessively unbalanced supply of nitrogen through the leaves and roots.

Simultaneously, it must be noted that foliar feeding with NPK, as compared with other experimental treatments, considerably increases the amount of aspartic acid [8], glutamic acid [12], alanine [15] and, particularly, tyrosine [17] and valine [19]. Feeding with phosphorus or potassium alone results in aspartic acid being synthesized in the greatest amounts, as compared with the other amino acids.

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CONCERNING BIOLOGICAL DIFFERENCES IN VARIETIES OF MAIZE

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In 1957, a comparative study was undertaken of the biological properties of two varieties of maize which differ in the length of the growing period (the early-maturing flint variety Beloyaroe psheno, and the medium-early starch variety Minnesota 13 extra).

In the end of July and in the first half of August, the transpiration rates of leaves of the two varieties were investigated under field conditions. For the determinations, pieces weighing about 450 mg were cut from the upper part of one of the later leaves and were at once weighed on a torsion balance, in the open. Weighings were repeated every minute four or five times. Leaves of the two varieties were always weighed alternately. This made it possible to compare the transpiration rate of pairs of leaves, one of each variety, differing in the time of determination within a limit of five-six minutes. Sixteen to twenty leaf pairs were taken for comparison every day.

The area of each leaf section was measured after weighing, and its absolutely dry weight was determined. Simultaneously, observations of temperature and air moisture were taken with the aid of an Assman psychrometer.

Investigation has shown that the two varieties differ in some characteristics of transpiration. As an example, in Table 1 results of transpiration determination are given for two days which differed in their weather conditions. Leaves of both varieties had approximately the same water content (75-77%) during the period of observation. During the hot sunny day of July 25, with a low relative air humidity and a great moisture deficit (Table 2), the variety Beloyaroe psheno used moisture more economically than variety Minnesota. In three minutes it transpired, on the average, 11.4% of the water present in the leaf. In the same time, the leaves of maize variety Minnesota used up 12.9%, or on the average 1.5% more. When pairs of leaves taken simultaneously are compared, the difference in water expenditure varied from −2.3% to +4.45%, in favor of variety Minnesota.

During the cooler day, with intermittent cloudiness, of July 27, with a high relative air humidity and small moisture deficit, the leaves of Beloyaroe psheno transpired more than on July 25. The large expenditures of water in leaves of this variety during the first minute indicates that their stomata are widely open. Water expenditure in variety Minnesota during the first minute is decreased, which may be taken as indicative of closed stomata.

The total transpiration in Minnesota leaves on July 27 was decreased, and in three minutes they spent, on the average, 9.8% of the water they contained, as compared with 13% used by leaves of Beloyaroe psheno, i.e., 3.2% less than the latter. Differences in the pair of leaves compared varied from −1.74 to −6.79%, in favor of variety Minnesota. Differences over five minutes were, on the average, 4%. In three minutes, the leaves of Beloyaroe psheno transpired 171.6 ± 6.74 mg of water per in 2 and leaves of Minnesota, 130.4 ± 5.44 mg, or 41.2 mg less. Expenditure for five minutes was 249.4 ± 6.02 and 202.0 ± 8.10 mg per in^2, respectively..

These differences in transpiration indicate different reactions of plants of the two maize varieties to a change in external conditions: during a hot day, with a soil water deficit, Beloyaroe psheno economizes on water, while it opens its stomata widely during a cool day; in Minnesota, stomata are opened wider during a warm day, and water evaporation is greatly increased, while during a cool day, transpiration in Minnesota is noticeably decreased.

TABLE 1

Transpiration of Water by Leaves of Two Varieties of Maize (in % of total amount of water in leaf)

Date of observation	Maize variety	Water content in total leaves, %	Water expenditure for the minutes				
			first	second	third	total in 3 min	total in 5 min
July 25	Beloyaroe psheno	75.2	4.55	3.56	3.31	11.42	–
	Minnesota	75.1	5.21	4.06	3.65	12.92	–
	Difference, in favor of Beloyaroe psheno		−0.66	−0.50	−0.34	−1.50	
July 27	Beloyaroe psheno	75.0	6.25	3.47	3.33	13.04	18.88
	Minnesota	76.8	3.98	3.07	2.74	9.79	14.90
	Difference, in favor of Beloyaroe psheno		+2.27	+ 0.39	+ 0.59	+ 3.25	+3.98

TABLE 2

Metereological Conditions during Observations of Transpiration in Maize Leaves

Date of observations	Average air temperature, C°	Relative air humidity, %	Moisture deficit, in millibars
July 25	22.8	51.8	14.3
July 27	18.6	74.4	5.9

Thus, it results that the transpiration rate in the two varieties of maize depends, to a great degree, on temperature, on its effect on the rate of the living processes of plants. While, within the limits of temperatures used during the period of the investigations, Beloyaroe psheno practically does not change its transpiration rate, Minnesota reacts relatively strongly to the temperature change. Relationship between temperature and transpiration is absent in Beloyaroe psheno (correlation coefficient is +0.232), while in Minnesota this relationship is quite noticeable (correlation coefficient is +0.632.)

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PHOSPHORUS AND IRON IN THE CELLS OF PHOTOSYNTHESIZING GREEN ALGAE

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Detailed accounts of the mineral chemical composition of plants have great significance for the study of photosynthesis, in connection with the discovery of the part played by phosphorus, iron and other elements in this process.

The first hypothesis of Ruben and collaborators [1] concerning the participation of energy-rich phosphorous compounds in the process of photosynthesis was confirmed by a number of other investigators [2-5]. According to the latest papers of Calvin, et al. [6], the initial reactions of photosynthesis lead to the formation of phosphor ylated, products, among which is found phosphoglyceric acid. The role of iron in photosynthesis also becomes more defined in the light of new data [7,8]. Investigations carried out by Boichenko and Zakharova [9] have shown that the initial products of photosynthesis contain phosphorus and iron.

In connection with questions of photosynthetic chemistry and with the fact that the chemical composition of fresh-water algae has been less studied than has that of marine algae [10], it seemed of interest to obtain data on the phosphorous, organophosphorous and iron content in the cells of green algae – Scenedesmus obliquus and Chlorella vulgaris – and to determine the effect of light on the amounts of these substances.

The question of the effect of light and oxygen on organophosphorous compounds in plants is debatable, and requires further study [3,4,5,11]. Because of this, we determined the organophosphorous content during anaerobic reduction by light of carbon dioxide in hydrogen, i.e., during photoreduction, in cells of Scenedesmus, and compared it with the content during photosynthesis. In this work, total iron was determined in the algae, in the light and in the dark, and total phosphorous, as well as the organophosphorous content (organic, acid-insoluble and unhydrolyzable phosphorus) was determined in the light under aerobic and anaerobic conditions, and also in the dark.

The cultivation of the algae and the carrying out of photoreduction has been described earlier [12,13]. The conditions of the experiments are described in Tables 1 and 2. Photoreduction was studied by means of hydroxylamine (1×10^{-2}M), which suppressed photosynthesis.

After light and dark experiments, cells were killed with liquid nitrogen and were divided into two groups: one group of cells was used for determination of phosphorus, the other, for determination of iron.

In the algae, determinations were made of organic phosphorus by the difference between total and inorganic phosphorus, of phosphorus remaining in the cells after extraction in trichloroacetic acid (acid-insoluble), and of unhydrolyzable phosphorus (three-hour hydrolysis in 1N HCl, according to Umbreit [14]), the greater part of which is composed of the phosphorus of phosphoglyceric acid. Iron in the algal cells was determined colorimetrically with potassium thiocyanate. The data obtained are given in Tables 1 and 2.

As can be seen from the data of Table 1, no significant difference is noted in the content of ash and iron in Scenedesmus cells under the influence of light.

TABLE 1

Content of Ash, Iron, and Phosphorus in Scenedesmus Cells (in % dry weight of cells; average of 6-7 determinations)

Process	Ash	Iron	Phosphorus			
			Total	Organic	Unhydrolyzable	Acid-insoluble
Photosynthesis	10.30	0.094	0.926	0.370	0.082	0.126
Photoreduction	10.20	0.095	0.922	0.362	0.076	0.128
Dark control	10.25	0.097	0.927	0.308	0.068	0.114

Note: Experimental conditions: illumination, ~ 2000 lux; temp., 25°, exposure, 2 hours; 4% CO_2 in gas phase; cells were placed in solution of $NaHCO_3$ (0.01 M).

TABLE 2

Content of Organophosphorous Compounds in Algae (in % absolutely dry weight of cells; average of 6-7 determinations)

Alga	Conditions	Phosphorus		
		Organic	Undrolyzable	Acid-insoluble
Scenedesmus	Light	0.374	0.083	0.120
The same	Dark	0.311	0.070	0.110
Chlorella	Light	0.480	0.112	0.149
The same	Dark	0.404	0.090	0.138

Note: Experimental conditions: illumination, ~ 6000 lux; temp., 25°; exposure, 2 hours; cells were placed in solution of $NaHCO_3$ (0.2 M).

Thus, the total iron content in algal cells is relatively constant under our conditions, but this does not exclude a possible change in its forms. It is quite possible that there exists in algal cells a constant, dynamic equilibrium of Fe^{++} and Fe^{+++}, as has been shown for higher plants [7]. Both in the content of total iron and total phosphorus, Scenedesmus cells in light do not differ from those in the dark (Table 1). The content of organic phosphorus in the cells of Chlorella and Scenedesmus, however, is greater in the light than in the dark (by approximately 15%). A somewhat smaller difference is found in the amounts of unhydrolyzable and acid-insoluble phosphorus under these conditions (Table 2). Consequently, some amount of cell phosphorus is drawn into phosphate changes under the influence of light.

It should be noted that the algal cells contain a large percentage of acid-insoluble phosphorus (~30% of the organic phosphorus).

When the amounts of organophosphorous compounds in Scenedesmus cells during photosynthesis and photoreduction are compared, it is seen that they are present in nearly equal amounts in these two processes, and in greater amounts than in the dark. The somewhat smaller amounts or organic phosphorus found during photoreduction than during photosynthesis agree with the smaller amounts of carbon dioxide absorbed during photoreduction, which we obtained earlier [13]. Thus, even during anaerobic reduction of carbon dioxide in hydrogen, phosphorylation takes place in algae without molecular oxygen, as it evidently takes place in bacterial anaerobic photosynthesis [15]. However, this does not exclude the possibility of using oxygen from the interior resources of the cell for phosphorylation during photoreduction, considering the different oxygen metabolism of the cell during photosynthesis and photoreduction [13].

The total amount of organophosphorous compounds in the cells of the two algae shows that the Chlorella cells are distinguished by a higher content than those of Scenedemus.

In conclusion, it should be noted that no difference was found in the total amount of iron and phosphorus in Scenedemus cells in the light and in the dark, but an increase in organic phosphorus was found in the cells of Chlorella and Scenedesmus under the influence of light (under aerobic and anaerobic conditions), which indicates the participation of phosphorus in the process of carbon dioxide reduction during photosynthesis.

The present work was carried out at the suggestion of E. A. Boichenko, to whom the author expresses deep gratitude for valuable advice and suggestions.

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PHOTOSYNTHETIC PRODUCTS INSOLUBLE IN ETHANOL AND WATER

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When studying the sequence of formation of the products of brief photosynthesis, most investigators analyze the compounds soluble in ethanol and water. At the same time, it is known that during photosynthesis CO_2 may enter into polysaccharides of the dextrin type, starch [1], polygalactose [2], proteins [3,4] and lipids [5]. In these works, only individual high-molecular weight compounds were studied, mostly not soluble in alcohol and water. Only in the work of Clendenning and Gorcham [6] was an attempt made to clarify the sequence of formation not only of low-molecular weight, but also of all high-molecular weight compounds. It turned out that the earliest compounds to be labeled in the photosynthesizing cells are free low-molecular weight compounds than lipids and "colloids" of chloroplasts, then the cytoplasmic proteins and cell walls.

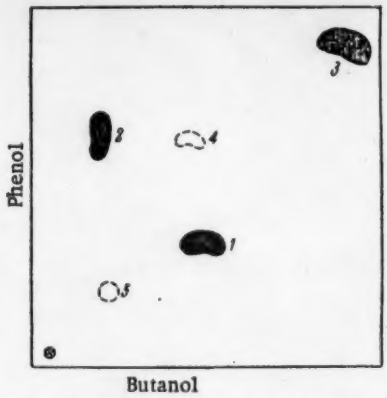
Our problem consisted in studying the formation of the products of brief periods of photosynthesis which are insoluble in ethanol and water.

Spring hard wheat, variety Gordeiforme 496, in the boot stage, was used as an experimental subject. The duration of photosynthesis for the formed leaves of the two upper nodes in a $C^{14}O_2$ atmosphere was one and five minutes. The concentration of CO_2 in the photosynthesis chambers was held at 0.5%, using Smis buffer (see [7]), and of $C^{14}O_2$, at 0.1%. Leaves (0.7g) were killed with boiling ethanol, then ground and washed consecutively with 80% boiling ethanol, hot 30% ethanol and water until no radioactivity could be detected in the wash liquid. Thus, the leaf material was divided into two fractions. We have published earlier [8] concerning the sequence of formation of the soluble products of photosynthesis.

Analysis of the insoluble material was done in the following way. The residues of five samples were combined into one lot of insoluble material. There were eight such lots for each period of photosynthesis of leaves. The lot of insoluble material was subjected to hydrolysis with 6 N HCl at a temperature of 115-125° for 24 hours. The hydrolyzate was separated from the residue and was evaporated with water several times for removal of hydrochloric acid. The products of hydrolysis were studied by means of two-dimensional chromatography on paper. Butanol, formic acid and water, in a ratio of 75:13:12, and phenol saturated with water were used as solvents. Radioautographs were taken from the chromatograms (exposure time of x-ray plates was two months).

The nonhydrolyzed residue was washed consecutively with 6 N HCl and water until disappearance of radioactivity in the wash liquid, dried and ground, after which the radioactivity of the residue (cellulose) was calculated, with a correction for self-absorption.

The radioactive carbon in the hydrolyzate was found in glyceric acid, asparagine, alanine (traces), aspartic acid (traces), and saccharic acid (see figure). Glyceric acid is formed, evidently, from substances of a lipoid type, since the latter give glycerine during hydrolysis, and under the conditions of our experiment glycerine is previously oxidized to glyceric acid. Arginin, alanine and aspartic acid are products of protein hydrolysis. Formation of saccharic acid under the conditions of our experiment may be represented in the following way: starch→glucose→saccharic acid. This is confirmed by treatment of standard starch and glucose with 6 N HCl at a temperature of 115-125° for 24 hours, as a result of which they are converted into saccharic acid. The fact that starch is intensively labeled during one-minute and five-minute photosynthesis of wheat leaves in an atmosphere of $C^{14}O_2$ was also confirmed by hydrolysis of the alcohol- and water-insoluble residue with salivary amylase.



Products of hydrochloric acid hydrolysis of high-molecular weight compounds of leaves. Compounds: 1) glyceric acid; 2) arginine; 3) saccharic acid; 4) alanine; 5) asparatic acid.

Distribution of C^{14} among Compounds Insoluble in Ethanol and Water (in % of total radioactivity of the insoluble-compound fraction)

Compound	Period of photosynthesis	
	1 min	5 min
Lipoids	39.3±3.7	9.5±1.4
Proteins	25.8±2.6	13.8±1.2
Starch	12.3±1.8	28.3±1.5
Cellulose	22.6±1.4	48.4±2.2

Measurement of radioactivity of the hydrolysis products (glyceric acid, arginine, saccharic acid) and cellulose (results of which are shown in the table) have shown that during one-minute photosynthesis most of the C^{14} was found in the glyceric acid (i.e., in substances of a lipoid type). Radioactive carbon was found in smaller amounts in arginine (i.e., in proteins), and in still smaller amounts in cellulose and starch.

After five-minute photosynthesis, the ration of radioactivity of the separate compounds was changed. The greatest radioactivity now is that of cellulose, next, that of starch. The least radioactivity was found in proteins and lipoids.

The results obtained allow us to insist that the carbon from $C^{14}O_2$ in the fraction of compounds insoluble in water and alcohol is first of all incorporated into the composition of lipoids, then into proteins, and last of all, into cellulose and starch. However, while cellulose and starch are formed last, the scale of carbon incorporation into them is greater than into lipoids and proteins.

It is interesting that, among compounds which are alcohol-soluble [9], water-soluble [10,11] and insoluble in water and alcohol (our experiments), the carbon from $C^{14}O_2$ is found first of all in the derivatives of glyceric acid. In the experiments of Calvin [9], it is found first in phosphoglyceric acid, in those of Boichenko and Zakharova [10,11], in polyglyceric acid, bound with atoms of phosphorus and iron.

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EFFECT OF SHORT DAY ON THE FORMATION OF GENERATIVE ORGANS IN LONG-DAY VARIETIES OF BLACK CURRANT

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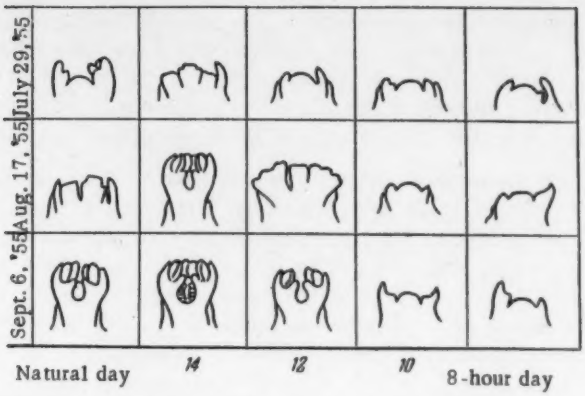
The differentiation of growing points and the development of the floral organs in apple, pear, cherry, wild-cherry, purple plum, red plum, apricot, peach, almond, currant, citrus, and grapes under naturally occurring long days in one area or another has been investigated by a number of authors [1-11 et al.]. However the effect of short or long days not indigenous to a given region, and consequently produced experimentally, on the formation of generative organs in fruit-berry crops has, as yet, hardly been investigated [12-13]. Furthermore, perception of this problem is of great theoretical as well as practical significance. The latter is especially important in the introduction of plants from one of the regions where they grow into other regions. The present paper contains the results of one of the experiments concerned with this problem.

For the past twenty-five years more than forty varieties and forms of black currant of different ecological-geographical origin, as well as native northern examples of this crop, which were obtained from below Igarka in the middle thirties and were well naturalized on the Kola penninsula, have been studied at the Polar Experimental Station of the All-Union Institute of Plant Culture (Murmansk region, Khibiny Station, 67°43' north latitude).

Long range observations on long day varieites of black currant revealed that it is very valuable for direct use in production as well as selection for frost-resistance and early ripening. Plants of Igarsky currant wintered well under the environmental conditions in the Murmansk region. For example, during the entire investigation, not once was there any freezing noted, at the station, of one-year-old shoots on this form. The average percentage of yearly loss of shoots on the one-year's growth consisted of 2.8%, whereas in the productive variety Liia, which was raised at the station which was standard for the northwestern regions, this loss was 35.9% (the percentage loss of the one-year-old shoots due to freezing was 19.6%). Igarsky currant plants begin to grow early (at an average daily air temperature of plus 1-3°). Under the conditions at Khibiny the berries began to ripen about 85 days after the beginning of growth (end of July). The berries were distinguished by their size (up to 1.5-2 g apiece) and their fine flavor. The yield reached 2.5 kg berries per bush (in Liia the yield was only 500 g).

The Igarsky form significantly surpassed all the other forms of black currant studied at the station in a number of characteristics. From a number of the best plants of the Igarsky currant, the first standards of this valuable crop, which has practically not been cultivated here earlier, were chosen for the Murmansk region.

Since the problem of frost resistance in black currant still remains for most of the regions where this crop is distributed, the qualitites noted in Igarsky currant give us a basis for recommending it as a specimen to be studied under experimental conditions, and to be used for breeding purposes in more southern regions, in contrast to the Murmansk region. This form of currant is of special interest for introduction into Karelo ASSR, Arkhangel'sk, Leningrad, and other regions in the northwest zone of the USSR. However, in relation to such an introduction this question arises: how will the plant react to the change in day length? In order to answer this question more accurately, we set out to establish the so-called lower critical day length for the given form of black currant. With this object in mind we performed some special investigations in 1955-1956. A day length



The effect of shortening the day to various day lengths on the initiation of reproductive organs in Igarsky black currant.

The Effect of Day Length on the Formation of Generative Organs in the Igarsky Form of Black Currant

Variants of the experiment	Beginning of differentiation in apical meristem of flower buds in 1955	Number of buds with differentiated apical meristem (from 10 buds examined in each variant)									Percentage of shoots with flowers based on the total number in the annual and biennial shoots (June, 1956)
		July 27	August 8	August 18	August 28	Sept. 6	Sept. 17	Sept. 27	Oct. 6	Oct. 12	
Natural day (control)	Variants of the experiment	0	0	9	10	10	10	10	10	10	71.5
8-hour	Not begun	0	0	0	0	0	0	0	0	0	0
10-hour	Not begun	0	0	0	0	0	0	0	0	0	0
12-hour	August 7-9	0	9	6	0	10	0	10	5	0	4.2
14-hour	July 27-29	8	0	10	8	10	0	9	10	0	9.6

of 8, 10, 12, and 14 hours was set up with experimental plants of Igarsky black currant. Midday was taken as the middle of the light period. We used plants which were growing at natural polar days typical for the location of the station for controls, (at the end of May – 23 hr, in June – 23 to 24 hr, in July – 24 to 20 hr, in August – 19 to 15 hr, during the first half of September – 15 to 13 hr). The days were shortened by covering the plants with chambers covered with tar paper. The inside volume of the chambers was one meter. In order to lower the inside temperature of the chambers they were painted white on the outside.

The experiments were begun on May 29, 1955 and lasted until September 16, inclusive. In each variant the experiment was set up on one plant. At the beginning of the investigation all the experimental plants were eight years old.

Observations made during the growing period showed that in spite of the fact that vegetative growth began at the same time, flowering of the plants kept at 8-to 14-hour days began 2 to 4 days later than in the control plants. The flowering period of these plants was 4 to 5 days shorter than in the control plants. However, shortening the day length artifically had an especially great effect on the initiation of new reproductive organs.

Beginning on July 29, that is two months after the beginning of the experiments, and every 10 days until October 12, inclusive, we made microscopic observations on longitudinal sections of flower buds in order to ascertain the effect of various daylight periods on the differentiation of the growing points. Each time 10 buds picked from year-old shoots in each variant of the experiment were examined. Some of the results obtained are given schematically in the figure.

Observations revealed that differentiation of the growing points began earliest in the flower buds of year-old shoots in plants exposed to a 14-hour day (see table). On July 29th greatly elongated protuberances and cylindrical formations were observed in these plants (the first phase of meristem differentiation), whereas the growing points in the control plants and the other variants were still undifferentiated (smooth). When the sections were examined on August 8, differentiation of the growing points had ceased in the plants from a 12-hour day. In plants from a natural polar day, differentiation of the growing points had just begun on August 16 to 18.

Hence these experiments showed that in plants from a 14-hour day, differentiation of the growing point in the flower buds on year-old shoots began about 20 days earlier than in plants from a natural polar day, and about 8-10 days earlier in plants from a 12-hour day. Consequently, when the day was shortened to 12-14 hours there was an acceleration in the development of reproductive organs in Igarsky black currant. This acceleration was also observed in later stages of floral organ development. However, differentiation of the growing points was by no means observed in all the side shoots of the plants in the 12-hour day (see table). When longitudinal sections of the buds were examined on August 18, differentiaion was observed in only 6 out of 10 of the buds. On August 28, September 17, and October 12, no differentiation of the growing point was observed in any of the 10 buds examined each time. On October 6, flower initials had appeared in only five of the 10 buds examined. Buds with flower initials were observed considerably more frequently in the plants from the 14-hour day. But on August 8, September 17, and October 12 the growing points in the 10 buds examined each time were not differentiated. As the experiments showed, differentiation of the apical meristem was observed more frequently in only the more developed flower buds of the middle-lower parts of the annual shoots. The apical meristem was usually not differentiated in the buds located in the upper parts of the year-old shoots. No differentiated was observed in the apical meristem of the buds in the weak annual shoots or in most of the reproductive shoots. This conforms with the observations made during flowering in 1956. For example, there were only single clusters on the plants from the 12-hour day. There were considerably more clusters on the plants from the 14-hour day (see table). The experiments showed that in plants growing in 8- and 10-hour days no differentiations occurred within the meristem of the flowering shoots in the fall of 1955.

Hence, on the basis of the experimental results presented above we can consider that the 12-hour day was apparently, a limiting (critical) day length during which plants of Igarsky black currant were still capable of developing generative organs. A 14-hour day was more favorable for this. Consequently, the introduction of Igarsky currant into Karelo ASSR, Arkhangel'sk, Leningrad, and other regions of northwestern USSR is completely possible since the day length in the indicated regions during spring, summer, and fall exceeds 14-17 hours.

The fact that apical meristem differentiation in flower buds of Igarsky black currant can occur even at a 12-hour day (without considering the 14-hour day) indicates that this form of currant is apparently not found as a native of the lower Yenesei region, but obviously comes from the more southerly regions and was introduced naturally into this region comparatively recently. However, this problem requires further special investigation.

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USE OF HETEROAUXIN TO STIMULATE POLLEN GERMINATION AND GROWTH OF THE POLLEN TUBES IN SOME FRUIT-BEARING CROPS

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Productivity and yield are closely related to the viability of pollen grains and the conditions necessary for their germination. The greater the viability of the pollen grains and the better the conditions for their germination, then usually, with all other conditions remaining equal, the greater the yield of the plants pollinated with this pollen [1-3]. Therefore, productivity and yield can be controlled by pollination, especially if conditions are favorable for pollen germination.

Pollen germination and the growth of pollen tubes, as well as other physiological processes can be stimulated by various means [4-9].

Favorable results were obtained when indolylacetic acid was used to stimulate pollen germination in several species of herbaceous plants [10, 11].

We investigated the stimulating effect of indolylacetic acid (heteroauxin) on the pollen grains of woody plants.

The work was done during 1955-1956 at the department of Darwinism and Plant Physiology at the Voronezh State University, and partially at the Botanical Garden of VGU.

Experimental material consisted of pollen grains from common cherry, variety Gremyamchenska (Cerasus vulgaris Mill.), Bessi cherry (C. besseyi Sok-Prunus Besseyi Bail.), domestic apple, variety Antonovka 600 gram movskaya and Pepin shafrannyi (Malus domestika Borkh. s. l.), garden pear, varieties Bere Zimnayaya Michurina, Bergamot Novik, and Bessemyanka (Pyrus communis var. sativa D. C. s. l.), common quince varieties Azerbaidzhanskaya Rea, Portugal'skaya Beretskii, and Shabran (Cydonia vulgaris Pers.).

Pollen from cherry was obtained in various ways: part of it was prepared from cut branches kept in containers of water in the laboratory, another part was obtained from growing specimens. Apple and pear pollen was obtained from growing trees. Quince pollen was ordered from the Nikitsky Botanical Garden [Yalta and from the Kubinsk Horticulture Experiment Station (Azerbaidzhanskaya SSR)].

A potassium salt of heteroauxin produced at the Moscow State University was used as a stimulator.

After it was picked, the pollen was kept constantly in parchment envelopes over sulfuric acid in a desiccator. Using the hanging drop method, the pollen grains were germinated in a glass chamber on glass microscope slides kept in an incubator for 24 hours at a temperature of 25-27°. The experimental media consisted of a 7.5% solution of glucose and distilled water with a dilute concentration of heteroauxin, 1:50,000, and with a greater concentration, 1:10,000. An uncontaminated 7.5% water solution of glucose served as the control. The pollen was sown with an innoculating needle. The experiments were performed in duplicate. When a mixture of various pollens was prepared, they were used in approximately the same ratio.

The pollen grains were counted, their viability determined, and the length of the pollen tube measured in four visual fields of the microscope (10 x 8). The length of the pollen tubes was measured in all of the germinated grains.

The results were brought together in a table.

From the table one can see that heteroauxin in a concentration of 1: 50,000 stimulated the germination of the pollen grains and the rate of growth of the pollen tube in cherry, pear, apple and quince. In connection with this, we made some field studies in 1956 in which we studied the role of heteroauxin as a stimulator in some hybridization work with the object of overcoming the incompatility of some forms. These preliminary experiments proved to be encouraging. When 358 apple flowers were pollinated with a mixture of apple, pear, and quince pollen without heteroauxin treatment there were no positive results; when 285 flowers were pollinated with this same mixture with heteroauxin, we obtained 10 ripe fruits from which 20 hybrid seeds were recovered. The method used in these experiments was as follows: after pollen was introduced on the mouth of the pistils with a rubber duster, they were dusted immediately with hereroauxin-talc mixture (1 g talc to 6 mg potassium salt of heteroauxin). A water solution of heteroauxin in the same concentration can be used in place of the powdered heteroauxin

The Effect of Heteroauxin on Pollen Germination and Growth of the Pollen Tubes in a Glucose Solution (7.5%).

Name of plants	Manner and date of pollen obtained	Heteroauxin concentration	Number pollen grains counted	No. of germinated pollen grains, %	Average length of pollen tubes, microns
Common cherry (one variety)	Cut branch, May 5, 1956	Control	78	10.3	490
		1:50,000	45	24.4	515
		1:10,000	115	10.4	387
	Growing tree, May 22, 1956	Control	162	0	0
		1:50,000	65	4.6	473
		1:10,000	157	1.9	443
Bessi cherry	Cut branch, April 5, 1956	Control	156	5.4	451
		1:50,000	101	20.8	530
		1:10,000	119	1.7	432
	Growing specimen, May 22, 1956	Control	122	4.9	443
		1:50,000	147	9.5	522
		1:10,000	131	2.3	438
Domestic apple (two varieties)	Growing trees, May 22, 1956	Control	225	0	0
		1:50,000	173	11.5	716
		1:10,000	290	0.3	Tube fell off
Orchard pear (three varieties)	Growing trees, May 22, 1956	Control	267	0	0
		1:50,000	171	21.6	908
		1:10,000	132	3.9	282
					Four of the five tubes dropped off
Common quince (five varieties)	Growing trees, May 13, 1956	Control	234	3.8	353
		1:50,000	175	22.7	1325
		1:10,000	231	1.3	795
Mixture of apple, pear, and quince pollen (ten varieties in all)	Growing trees, May 13-22, 1956	Control	338	2.7	325
		1:50,000	348	38.5	1037
		1:10,000	215	0.5	Tube fell off

Note: Common cherry and Bessi cherry pollen was applied June 7, all the other varieties were applied June 4, 1956.

We found that in most cases more concentrated hereroauxin (1:10,999) did not have a stimulating effect when the pollen was treated with it, but acted as a herbicide. Its herbicidal properties showed up very clearly when apple and pear pollen was treated; not only was pollen germination and growth of the pollen tubes inhibited, but their destruction was also observed; this expressed itself particularly in the falling-off of the still not definitely formed pollen tubes from the pollen grains.

It is interesting to note that in spite of the mutual stimulation, a mixture of apple, pear, and quince pollen not treated with heteroauxin produced better results in respect to germination and pollen tube growth even in relation to similar pollen from apple and pear, but it yielded finally in this respect to quince pollen.

This can be explained by the fact that in our experiments quince pollen without heteroauxin treatment was much more viable than it was when used as a component of a mixture.

However, the use of heteroauxin considerably improved germination not only of homegeneous pollen but also of a pollen mixture. For example, in the control, the total germination of a pollen mixture from apple, pear, and quince was 2.7% and the length of the pollen tubes was 325 microns, but when this same mixture was treated with a potassium salt of heteroauxin germination consisted of 38.5% (i.e., it was 14.2 times greater than the control), and the length of the pollen tubes was 1037 microns (i.e., 3.2 times that of the control).

It was also shown in our experiments (see table) that the viability of cherry pollen which had developed on cut branches in containers of water not only was not reduced, but sometimes exceeded that of the pollen obtained from growing specimens; whereupon treatment of such pollen grains with a weak heteroauxin solution greatly stimulated germination as well as pollen tube growth.

Therefore, low concentrations of the potassium salt of heteroauxin stimulate the germination of pollen from various varieties of several woody fruit-bearing crops and can be used in hybridization experiments for overcoming the incompatability of forms separated phylogenetically.

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VEGETATIVE GROWTH OF THE INFLORESCENCES OF VARIOUS PLANTS IN RELATION TO THEIR OXIDATION-REDUCTION ACTIVITY

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Biological literature contains reports concerning instances of vegetative growth in inflorescences. Therefore from data in the literature and our own experiment [1] we are aware of instances where inflorescences have reverted to vegetative organs in 109 species from 23 families. However, the physiological-biochemical nature of the vegetative growth of inflorescences has remained unclarified since the investigations by Klebs.

Studying the oxidation-reduction (o-r) processes of plants in relation to photosynthesis Krasinskii [2,3] came to the conclusion that the transition of plants from the vegetative to the flowering stage is related to the o-r processes of the growing tissues. He proposed that the reversal of the inflorescence to vegetative growth is also associated with the oxidation-reduction processes. These considerations served as a starting point for our investigators.

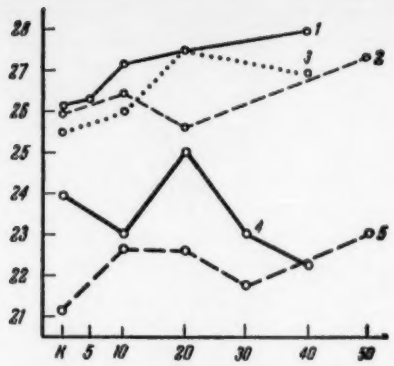
Potatoes were the principle material, used. In addition, we used two species, which, like potato, also exhibit reversal to vegetative growth in the inflorescence: speedwell (Veronica chamaedris L.) and figwort (Scrophularia nodosa L.) For controls we used two species of plants which did not exhibit any reversal to vegetative growth in the inflorescence in our experiments: common speedwell (Veronica officinalis L.) and loosestrife (Lysimachia vulgaris L.).

Vegetative growth of the inflorescence was obtained by using flowering cuttings, i.e. cuttings with inflorescences which were kept in the shade after they had rooted (for greater detail see [1]). In addition we also obtained this transition, in potato, on the entire plant by disbudding, i.e., carefully removing the axillary buds on a plant with a single shoot.

The oxidation-reduction processes were characterized by the o-r potential values (Eh) and the aerobic index (rH). The Eh and pH were determined by the electrometric method according to the compensation process using platinum not platinum plated electrodes (with modifications by N.P. Krasinskii) emmersed in a paste obtained by grinding the tissues of the experimental plants. The Eh value is given in volts. Determinations were made for the leaves and inflorescences of the flowering cuttings on the day they were cut, and then again 5, 10, 20 and 40 days after cutting. Experiments were performed in 1947, 1948 and 1949.

The data obtained (Table 1) show that according to Eh and rH values the plants studied could be divided into two groups. First group – the plants which exhibited reversal (potato, speedwell, figwort). Their Eh and rH values were comparatively high. Second group – the plants which did not exhibit reversal (common speedwell and loosestrife). In plants of the second group the Eh and rH values were on an average, considerably lower than in the first ones.

Since the pH of plants which exhibited reversal was higher than that of the plants which did not exhibit reversal, the difference in the rH values of both groups of plants was considerably more defined than in the Eh values. This was true for the leaves as well as the inflorescences. These data permit one to make a preliminary conclusion that in flowering cuttings from plants which exhibited reversal, the oxidation processes are greater than in flowering cuttings which do not exhibit reversal.



A comparison of the changes in the aerobic index (rH) in the leaves of various plants. The rH values are plotted on the ordinate, the number of days after cutting on the abscissa; 1) potato; 2) speedwell; 3) figwort; 4) common speedwell; 5) loosestrife.

There were no clear differences disclosed between the plant species which exhibited reversal and those which did not in the rate of the change of the o-r process during the experiment. Nevertheless, on the basis of the experiments presented one can purpose that flowering cuttings of potato, speedwell, and figwort, i.e., species which exhibited reversal, when cultured under conditions of deep shade, begin to increase in Eh and rH values. Vegetative cuttings of potato behaved like flowering cuttings. In the flowering cuttings of species which did not exhibit reversal, the shift in the o-r processes toward oxidation was weaker than in plants which exhibited reversal, or else this shift was even directed toward reduction (see figure).

In order to compare the o-r processes of cuttings bearing developed reversal shoots with that in cuttings without such developed shoots, a special experiment was set up in 1949. The changes in Eh and rH values were traced in the leaves of one and the same cuttings during the growth of the reversal shoots. Controls consisted of cuttings with reversal shoots on which the buds of the reversal shoots were removed.

TABLE 1

Comparison of Average Eh, pH and rH Values in Plants Which Exhibited Reversal and Those Which Did Not

Experimental material	Leaves			Inflorescences		
	Eh	pH	rH	Eh	pH	rH
Potato	0.462	5.49	26.95	0.498	5.20	27.63
Speedwell	0.445	5.58	26.37	0.470	5.65	27.38
Figwort	0.424	5.94	26.51	–	–	–
Average	0.444	5.67	26.61	0.484	5.43	27.51
Common speedwell	0.377	5.26	23.49	0.428	5.41	25.37
Loosestrife	0.380	4.66	22.29	–	–	–
Average	0.379	4.96	22.89	0.428	5.41	25.37

TABLE 2

Average Eh, pH, and rH Values in Flowering Potato Cuttings Which Were Made at Different Stages of Flowering

Variants of experiment	% reversal	Leaves			Inflorescences		
		Eh	pH	rH	Eh	pH	rH
First half of flowering	64.6	0.445	5.67	26.58	0.454	5.40	26.36
Second half of flowering	27.7	0.429	5.69	26.14	0.449	5.65	26.67
End of flowering	6.6	0.471	5.52	27.35	0.521	5.13	28.40

During the first period of growth of the reversal shoots the Eh value increased from 0.383 to 0.402 and the rH from 24.31 to 24.70. In the control, cuttings were directed in another direction – toward reduction.

TABLE 3

Relation of O-R Activity in Potato Leaves to Disbudding and the Number of Leaves on the Stem

Experimental variants	Eh	pH	rH
Control	0.422	5.66	25.71
Disbudded, one leaf	0.393	5.62	24.63
Disbudded, three leaves	0.357	5.99	23.64

The following hypothesis was made on the basis of the results obtained. Reversal begins to occur during the period when the o-r processes in the flowering cuttings shift toward oxidation. However, in order to obtain maximum reversal a somewhat average (optimal) o-r activity is necessary. In order to verify this, experiments were set up in which it was possible to change the o-r activity of the flowering cuttings or the entire potato plants by establishing different conditions in the various variants. Proceeding on our hypothesis, we expected to obtain a greater reversal in the variants with an average oxidation activity than in those with a maximum oxidation activity.

TABLE 4

The Change in Carbohydrate Content of Cuttings with Flowering Inflorescences from Potato, Variety Lorkh (in % absolute dry weight)

Carbohydrates	Number of days after cutting					
	0	5	10	20 days without reversal shoots	20 days with reversal shoots	40
Total sugars	10.87	8.75	6.47	7.06	9.06	7.06
Starch	3.10	3.25	1.32	1.77	2.10	1.21
Total carbohydrates studied	13.97	12.00	7.79	8.77	11.16	8.27

In 1948 six experiments were set up with flowering potato cuttings of variety Lorkh. The cuttings were made at the beginning, middle and end of flowering. A comparison of the data obtained is given in Table 2.

From the data in Table 2 it is evident that on the basis of o-r activity, a third group (the end of flowering) in which the greatest oxidation activity was observed differs even more from the first two groups; in addition a minimum degree of reversal was observed in the cuttings of the third group. This makes it possible to consider that an excessive shift in o-r activity of the flowering cuttings toward oxidation is associated with a decrease in the degree of reversal; this agrees with the hypothesis mentioned above.

In 1949 an experiment was set up with three variants using intact potato plants (see Table 3). In the last variant two leaves were left on each reversal shoot, then their axillary buds and the terminal buds were removed. Consequently, three leaves originated at each node of the main stem. The variations in leaf area, unequal growth of the shoots, and the irregular use and translocation of assimilates resulted in a variation of o-r activity of the leaves within the variants, apparently, primarily, due to the variation in their sugar content. The average values in 8-25 leaves from different variants of the experiment are given in Table 3.

As we can see from Table 3, the average o-r activity was in the second variant; in the first variant it was displaced to the oxidation side, and in the third one it was displaced to the reduction side.

The maximum degree of reversal occurred in the second variant (29.6%), in the first one it was one half as great (14.3%), and in the third it was three fourths as great (19.4%). Even though only one experiment was performed, the data obtained permit as to conclude that in order to obtain the greatest degree of reversal a somewhat average (optimal) o-r activity is necessary.

This conclusion agrees with the theoretical hypothesis of the experiment.

The above-mentioned hypothesis also agrees with Sinyukhin's data [4], which showed that the development of callus occurs at high Eh and rH values since the initiation of secondary meristems (appearance of additional buds) favors a more reduced o-r activity, which in his experiment was produced in anaerobic conditions.

As a preliminary study of the relationship between carbohydrate metabolism and the reversal to vegetative growth of inflorescences in potato, including the relationship of o-r activity carbohydrate content, measurements were made of monosaccharide, disaccharide, and starch content of the leaves and inflorescences of flowering potato cuttings. The data obtained with inflorescences of potato cuttings using variety Lorkh prepared in 1948 are given in Table 4.

As we can see from Table 4, when the cuttings grew at a weak illumination their carbohydrate content gradually decreased. However, in the cuttings where reversal had occurred, 20 days after the cuttings were made the total of the investigated carbohydrates was 2.39% greater than in the cuttings in which reversal had not occurred. Therefore, even though the reversal to vegetative growth of potato inflorescences occurred after the carbohydrate stores had decreased (in cuttings where reversal had occurred they were less than at the time of cutting), reversal occurred only in those cuttings where the decrease in carbohydrate content was not excessive.

There is a close relationship between o-r activity of plants and their carbohydrate metabolism [5]. Oxidation-reduction systems with low Eh and rH values form sugars. For that reason the data concerning carbohydrate content of flowering potato cuttings agree with the above-mentioned hypothesis.

Thus, the reversal of inflorescences into vegetative shoots occurs more readily in plant species having a greater oxidation activity of the o-r system. Furthermore, a moderate shift of o-r activity toward oxidation is necessary; this is associated with a decrease in carbohydrate content. However, an extreme decrease of carbohydrates and an excessive shift of o-r activity toward oxidation also inhibits the appearance of such transformations.

Considering the relationship between the reversal of inflorescences and the condition of o-r activity of flowering cuttings, one should note that the facts obtained can not be explained simply. The appearance of reversal to vegetative growth is of course, accompanied by complex changes in metabolism. These changes affect the o-r activity of the cuttings and at the same time depend on it to a considerable degree.

The present investigation was performed under the direction of Professor Nikolai Petrovich Krasinskii.

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*See English translation.

METHODS

QUANTITATIVE DETERMINATION OF CHLOROPHYLLS A AND B USING PAPER CHROMATOGRAPHY

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In considering the many physiological, biochemical and ecological problems, it becomes necessary to determine not only the total amount of green pigments in the plastids, but also the individual components.

For this kind of an experiment a chromatographic separation of these pigments is essential since an alcohol or acetone extract may also frequently contain chlorophyllides, in addition to the chlorophylls, which absorb light in the same region as the chlorophylls.

In a paper published earlier [1] we indicated that a separation of pigments using paper chromatography has several advantages over column chromatography. Therefore, we proceeded to work out a method for a quantitative determination of chlorophylls a and b using paper chromatography.

DESCRIPTION OF METHOD

Weighed portions (from one to two grams) of leaves were ground in an acetone-ethanol (96%) mixture (in a volume ratio of 3:1) with the addition of a small amount of soda or quartz sand. In order to rapidly inactivate the enzymes it was necessary to first fix the weighed samples in cold acetone at − 78° [2].

The paste obtained was filtered through a No. 2 glass filter ("Druzhnaya Gorka"); the sediment on the filter was rinsed two-three times with the same acetone-alcohol mixture; and the volume of the extract was brought to

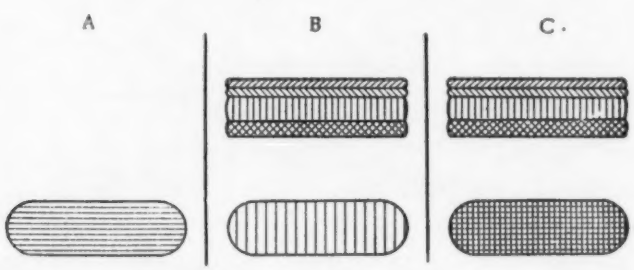

Diagram of the chromatographic separation of chlorophylls: A) chromatogram with the original spot; B) chromatogram after separation; no plastid pigments remain in the area of the original spot; the upper spot contains chlorophylls a and b, separated by a light strip (vertical lines); C) the same as B, but the chlorophyllides remained in the original spot.

TABLE 1

A comparison of the Amount of Chlorophylls a and b before and after Chromatography (in μg/ml)

Chlorophyll A		Chlorophyll B	
before chromatography	after chromatography	before chromatography	after chromatography
61.7	61.7	36.3	36.3
61.5	61.4	35.3	35.9
61.1	61.1	36.6	36.6
61.2	61.2	36.3	36.3
61.4	61.4	36.6	36.6
Average 61.4∓0.2	61.4∓0.2	36.2∓0.4	36.3∓0.2

TABLE 2

A Comparison of the Total Amount of Chlorophylls a and b before and after Chromatography (in μg/ml)

Total amount of chlorophylls A and B before chromatography	Amount of chlorophylls after chromatography		Total amount after chromatography (calculated)
	a	d	
44.6	32.8	11.6	44.4
44.0	33.2	10.6	43.8
44.0	32.7	11.6	44.3
43.6	32.7	10.6	43.3
43.6	32.0	11.3	43.3
43.6	32.8	10.6	43.4
43.6	33.2	10.1	43.3
Average 43.8∓0.3	32.8∓0.3	10.9∓0.5	43.7∓0.5

TABLE 3

Chlorophyll Content of a Leaf Extract from Cyclamen, Determined Directly, Photometrically and Chromatographically (in μg/ml)

Directly photometrically	After chromatography			Directly photometrically	After chromatography		
	chlorophyll a	chlorophyll d	total		chlorophyll a	chlorophyll d	total
Experiment No. 1				Experiment No. 2			
25.5	19.1	5.3	24.4	50,4	34,5	12,1	46.6
25.0	17.9	5.6	23.5	50,6	34,5	12.4	46.9
25.4	18.4	5.3	23.7	50,4	35.2	12.9	48.1
—	18.2	5.9	24.1	—	35.0	12.1	47.1
—	17.9	5.9	23.8	—	34.3	12.2	46.5
—	18.2	5.1	23.3	—	34.3	11.7	46.0
Average 25.3	18.3	5.5	23.8	—	35.3	11.7	47.0
				Average 50,5	34.7	12.2	46.9

a volume of 25 ml in a volumetric flask. Using a calibrated pipette, 1-2 ml of green extract was placed in a band across a strip of chromatographic paper measuring 13 x 16 cm. We used paper No. 1 (crab base). The paper was rolled up on a slant in the form of a cylinder 13 cm high and was placed in a vessel used for chromatography. A cylindrical glass jar with a ground-glass cover was used for this; it was 20 cm high, and 10 cm in diameter. Twenty milliliters of a petroleum ether-ethly alcohol mixture were placed into the jar first. The volume ratio of these solvents could vary from 20: to 1.0 to 20: 1.5.

TABLE 4

Amount of Chlorophyll a and b in the Leaves of Various Plants, Determined Using Paper Chromatography (in μg/g fresh weight)

Date of analysis	Name of plants	Weight of leaves, mg	Chlorophyll content	
			a	b
21.XI	Aspidistra	774	1420	524
		761	1540	564
		785	1410	561
		859	1470	577
		765	1520	579
		762	1390	496
		average	1460∓5	547∓3
26.XI	Ficus elastica	1445	649	245
		1432	710	262
		1461	707	265
		1442	655	239
		1433	664	241
		1466	624	237
		average	670∓27	248∓10
29.XII	Vitis Voyneriana	1175	664	252
		1185	665	248
		1156	660	245
		1149	685	—
		1144	668	—
		average	668∓7	248∓2
24.XII	Banana	510	1880	606
		516	1900	570
		527	1770	589
		554	1750	556
		557	1700	—
		546	1720	—
		average	1790∓70	582∓17

The separation of chlorophylls a and b occurred by means of the upward movement of the solvent mixture (see Fig. 1). The time required for the separation was 20-30 min. After complete separation of chlorophylls a and b had occurred, the paper was removed from the jar, strips with the corresponding pigments were cut out with scissors, and each strip was cut up into small pieces and placed into small chemical glasses.

At first the elution of chlorophylls was accomplished using small amounts of ethyl-ether; then the solution was washed into a 10 ml flask with an acetone-ethanol mixture. The optical density of the pigments was measured on a photoelectric colorimeter (FÉK-M). Carotenoids, present when the indicated method for the chromatographic separation of chlorophyll is used, do not interfere with a quantitative determination of chlorophyll since the optical density is measured with a red light filter.

In order to work out the method it was first of all necessary to clarify how stable the chlorophylls were during the conditions accompanying chromatographic separation. The following series of experiments was set up with this objective.

1. Solutions of chlorophylls a and b in a known concentration were placed separately on sheets of paper and chromatograms were made according to the method described above. After elution the pigments were measured photometrically. The results are given in Table 1.

2. A mixture of chlorophylls a and b in a known concentration, obtained by means of the preliminary chromatography of the green extract was placed on the chromatographic paper.

After chromatography of the mixture each of the pigments was eluted and individually measured photometrically. The results of these analysis are given in Table 2.

Results of the analyses given in Tables 1 and 2 show that the observed differences between direct photometric measurements and chromatography when we were using pure solutions of chlorophylls are within the limits of experimental error.

The investigation with the green extract was somewhat different. Table 3 contains a comparison of the results of the analyses of alcohol-acetone extracts from cyclamen leaves before and after chromatography.

As we see from Table 3, the total sum of chlorophylls a and b after chromatography was somewhat lower than that determined directly photometrically.

A study of the extract revealed that, in addition to the chlorophylls, it also contained chlorophyllides which remained in the original spot during chromatography (see Fig. 1, B). The optical properties of chlorophyllides are close to those of chlorophylls, therefore when measured with a photoelectric colorimeter(or, correspondingly, with a spectrophotometer) the optical density of the chlorophyllides was equal to the optical density of the chlorophylls. As a result, direct photoelectric measurements of the extract resulted in higher values.

The data obtained from chromatographic analyses of the chlorophyll a and b content in four species of plants are given inTable 4.

As we see from Table 4, the variation between parallel determinations does not, on an average, exceed 4.

SUMMARY

On the basis of the experiments described above we were able to draw a conclusion concerning the usefullness of the described method for measuring the amount of chlorophyll A and B in the leaves of green plants, since it was demonstrated that when paper chromatography was used there was neither any loss nor dissociation or chlorophylls a and b.

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EXPERIMENTAL VERIFICATION OF THE RADIOMETRIC METHOD FOR CALCULATING THE RATE OF PHOTOSYNTHESIS

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During an investigation of photosynthesis in plants it is extremely important to describe not only the course and rate of the processes involving synthesis, transformation, and translocation of the photosynthetic products, but also the quantitative direction of these processes. In order to do this it is necessary to bring about an equilibrium between the carbon absorbed by the plant from the external media and that found in the individual organs or organic compounds of the plant. However, it is only possible to attain such an equilibrium with a precise quantitative calculation of radioactivity in absolute units (mC) or units proportional to them (dissociation per min, counts/min of the preparation with correction for the background counts and decay) rather than in comparative units (counts/min from the preparation). This explains the special attention of investigators to the scientific methods for converting the number of counts/min into absolute quantitative measurements of radioactivity (mC, mg, or number of moles C^{14}, etc.). At the present time several such methods have been worked out.

In 1947, J. Reid [1] proposed a method for calculating the absolute activity of preparations containing C^{14} based on several theoretical conditions of curves, experimentally obtained, which characterize the dissociation and decay of β-particles in the substances through which they pass. This method is described in detail and worked out by Bochkarev and co-workers [2], Gusev [3], Spitsyn and co-workers [4], and others. But only a few investigators make use of this method; this can be explained by the great complexity of the theoretical calculation required and the difficulty of verifying the applicability of Reid's curves for other conditions of calculating radioactivity than in his experiments.

Several method manuals propose determining activity of preparations by comparing them with standards. But the preparation of standards is a special operation whose complexity at the present time limits the possibility of using this method for converting counts/min into absolute units. A method for measuring absolute activity which requires a complex vacuum apparatus has been developed in the physics laboratory. Because of the lack of such apparatus in most biological laboratories, and also, it seems to us, principally because of the impossibility of using it for solving many problems concerning carbon metabolism in plants, this method cannot be widely used for plant physiology purposes.

While working out means of using C^{14} for determining the characteristics of photosynthesis in plants in natural conditions, Voznesenskii [5] proposed a method for converting the number of counts/min of a preparation into milligrams absorbed CO_2; this requires comparatively simple preliminary measurements which can be made in general laboratories. This method is the basis for determining the ratio coefficient between the number of milligrams of CO_2 of a known specific activity which are absorbed by the leaves from the external environment, and the radioactivity (counts/min of the preparation with correction for background count and decay) of preparations prepared from exposed leaves. In order to verify these determinations a special chamber was made from plastic, provided with a β-counter, ventilation and refrigeration. A detailed description for determining the coefficient is given in the paper by Zalenskii and co-workers [6] and those of Voznesenskii [5, 7]. Having determined the ratio coefficient, and knowing the specific activity of the carbon dioxide used in the experiments, it is possible to calculate the amount of C or CO_2 which entered one or another organ of the experimental plant, or a specific organic compound isolated from the plant in which an investigator might be interested. It is necessary to note that when calculating radioactivity special attention is required in the preparation of samples (Spitsyn and co-workers.

TABLE 1

A Comparison of Photosynthesis Rates in Cineraria Leaves, Obtained by the Air-Flow Method and Radiometrically

No.	Rate of photosynthesis, mg CO_2/in^2 · hr		Ratio between measurements, T/R	No.	Rate of photosynthesis, mg CO_2/in^2 · hr		Ratio between measurements, T/R
	Air-flow T	Radiometrically, R			Air-flow T	Radiometrically, R	
1	28.95	31.3	0.925	26	18.9	18.0	1.050
2	16.5	19.1	0.865	27	18.15	18.0	1.008
3	42.0	31.0	1.350	28	13.2	12.8	1.030
4	19.8	24.4	0.812	29	16.4	16.0	1.025
5	19.2	29.2	0.660	30	26.6	27.2	0.978
6	21.0	20.3	1.035	31	12.7	13.1	0.970
7	15.9	25.1	0.633	32	15.9	21.5	0.740
8	17.4	22.3	0.780	33	19.5	19.8	0.985
9	21.7	22.5	0.965	34	13.2	10.5	1.257
10	20.1	23.5	0.855	35	20.7	24.8	0.835
11	19.8	24.2	0.820	36	15.9	11.6	1.370
12	20.8	19.5	1.065	37	16.8	24.0	0.700
13	15.3	15.2	1.007	38	32.5	27.7	1.174
14	22.8	21.3	1.070	39	33.5	32.3	1.035
15	23.1	23.3	0.995	40	28.5	26.0	1.095
16	19.2	23.4	0.820	41	40.0	38.0	1.050
17	11.4	11.4	1.000	42	40.5	39.0	1.040
18	14.1	12.3	1.145	43	12.9	13.0	0.993
19	18.6	17.3	1.075	44	11.3	11.6	0.975
20	15.3	14.5	1.055	45	28.8	42.0	0.686
21	17.7	17.5	1.010	46	24.7	24.9	0.993
22	20.4	19.8	1.030	47	8.4	8.0	1.050
23	20.1	18.1	1.110	48	8.4	7.3	1.150
24	33.0	20.8	1.585	49	34.0	37.5	0.910
25	15.6	14.2	1.095	50	35.5	46.0	0.773
					average		0.993±0.125

We verified the proposed method for converting counts/min into absolute units by comparing the rate of photosynthesis obtained using the radiometric method, which included a conversion of the number of counts/min into mg CO_2/in^2, hr, with measurements using the "air flow" method with the subsequent titrations of the absorbing alkali solution, or Warburg's manometric method. Such experiments were performed simultaneously using both methods on one leaf, or sections cut from it.

The apparatus for determining the rate of photosynthesis using the radiometric method and the air-flow method simultaneously consisted of a gas tank, leaf chamber, illuminating lamp with a water filter, galvonometer, carbon dioxide absorbent, and a gas meter. The gas tank consisted of a carbon dioxide cylinder, about 40 l in volume, filled with a mixture of air and radioactive carbon dioxide at a pressure of 3-4 atmos; the concentration of CO_2 was 0.5%, the specific radioactivity was 0.25 mC/l CO_2. The flow of air from the cylinder during the experiment was regulated with a reducing valve. During the assembly of the apparatus special attention was given to obtaining an air-tight seal between the individual parts, and also the correct operation of the valve on the gas cylinder. The gas from the cylinder entered the leaf chamber which consisted of a fused metal box with a removable top cover. The cover and the insertion piece were of plastic which was screwed to the edge of the chamber. In order to get an air-tight connection a rubber gasket was placed over the edge. Within the chamber were strung several strings on which the leaves were placed. Carbon dioxide was absorbed in Chesnokov's absorption apparatus (glass tubes 4 cm in diameter, 40 cm high, with 200 ml $Ba(OH)_2$, 0.025 N, diffuser-glass filter No. 2; absorption capacity was 98-99%). The galvanometer was installed after the absorption apparatus, in order to control the continuity of air flow, and a gas meter brand 1-GSB-400 to measure the volume of air which passed through the leaf chamber and the absorption apparatus. The barium solution was titrated with 0.025 N hydrochloric acid.

The order of operation was as follows: first, after the gas cylinder was filled with radioactive carbon dioxide (and also several times during the experiment) the actual concentration of CO_2 in the cylinder was determined. Then 1 in^2 of leaf area was placed into the chamber, the light turned on (about 20,000 lux), and a current

TABLE 2

A Comparison of Photosynthesis Rates, Obtained Manometrically and Radiometrically

No.	Plant names	Rate of photosynthesis, mg CO_2/ $in^2 \cdot hr$		Ratio between values, M/R
		manometric, M	radiometric, R	
1	Beans	10.33	9.6	1.07
2	Beans	13.5	13.5	1.00
3	Beans (old leaves)	4.24	4.54	0.935
4	Cineraria	16.4	16.2	1.015
5	Ficus Ben'yamina	6.4	6.3	1.02
6	Cineraria	13.4	14.0	0.96
			Average 0.998 ±0.034	

of air from the cylinder was passed through (20-30 l/hr). The experiment lasted 20 min. The amount of CO_2 absorbed by the leaf in the two experiments was calculated from the difference in the amount of hydrochloric acid used to titrate the barium hidroxide solution. Computing this amount for a leaf exposure of 1 hour, we obtained the rate of photosynthesis in mg $CO_2/in^2 \cdot hr$.

After exposure, the leaf was fixed in hot alcohol, dried, weighed, and ground to a powder from which the sample for measuring radioactivity was prepared. We converted from counts/min to mg CO_2, according to the method described above. Considering the leaf area and the duration of the experiment, we also determined the rate of photosynthesis in mg $CO_2/ in^2 \cdot hr$, on the basis of the radioactivity contained in the leaf itself. The results of the experiments are given in Table 1. In order to compare the methods at different rates of photosynthesis the work was purposely done on different plant material.

In order to do this, we picked cineraria leaves at different stages of growth at various times of the day, from different plants which had grown at somewhat different conditions. In some instances, photosynthesis was measured at various temperatures; for example, in experiments 43, 44 and 48 the temperature was from 4 to 5°, but in experiments 45, 46, 49, and 50 it was 26 to 27°.

A comparison of the data obtained by radiometric measurement of photosynthesis rate and those using Warburg's manometric method were made using an apparatus whose construction is described in the paper by Zalenskii and co-workers [4]. Good temperature control of the water bath, especially constructed manometric flasks made of plastic, illumination from below, the ability to produce any oscillation rate, and a thorough preliminary investigation to find the optimal conditions for photosynthesis in the leaf cuttings placed in the vessels, enabled us to get reliable data concerning the rate of photosynthesis under conditions of the experiment.

The source of carbon dioxide for the leaf was Warburg's carbonate-bicarbonate buffer No. 9 (0.1 M), which maintained a CO_2 concentration of about 0.3% in the atmosphere within the flask. The most important factor in a manometer measurement of photosynthesis rate is the necessity of maintaining the concentration of carbon dioxide in the flask at a constant level. This can only be attained if the rate of carbon dioxide absorption by the leaf cutting during photosynthesis does not exceed the evolution of carbon dioxide from the buffer solution into the atmosphere of the vessel. Therefore, for each sample that we used, the area of the leaf cutting was determined in special preliminary experiments.

A comparison of the values obtained for photosynthesis rates from both comparable methods was made during the constant rate of the process. At first the leaf photosynthesized without a radioactive buffer, this was later (after a period of 40 min to 2 hours) exchanged for a buffer having the very same properties but with radioactive carbon. The specific activity of carbon dioxide which evolved from the buffer was equal to 0.1 mC/l CO_2. After the light was turned on, when the flask already contained radioactive buffer, the manometer stopcocks were not closed immediately, but after 10 min had elapsed, during which time, as has been shown, equalization of the

temperature occurred and the gas phase of the vessel became saturated with carbon dioxide liberated from the buffer. At the same time, in the radiometric method, we recorded the time when exposure to radiation began from the time the light was turned on, and in the manometric method – from the moment the manometer stopcocks were closed. The duration of the experiments varied from 50 to 90 min. Illumination within the vessel reached about 30,000 lux. Controlled readings shown on the manometers were recorded every 15 or 20 min.

In this way we determined the rates of photosynthesis for each section; these were then added up and converted to 1 $in^2 \cdot hr$. When the exposure was completed all the leaf sections were fixed in alcohol vapor, dried, and weighed. Samples, prepared from 8-10 pulverized sections, were checked with a face β-particle counter and the results obtained were converted to milligrams carbon dioxide absorbed by 1 in^2 leaf area in 1 hour. In order to compare this with the data obtained using the manometric method, the photosynthesis coefficient (ratio between volume of carbon dioxide absorbed and the volume of oxygen evolved) was taken as one. Therefore, a comparison of photosynthesis rates obtained radiometrically with those obtained manometrically was made under the same experimental conditions. The data obtained are given in Table 2.

In order to fully evaluate the results obtained it was necessary to visualize the accuracy of each method which was used to calculate the compared values. The error in determining the rate of photosynthesis using the air-flow method is due primarily to the accuracy of measuring the volume of air passing through; in our experiments this was as high as 17%. The principal source of error in a manometric determination of gas exchange is an uneven temperature within a temperature controlled bath. In the present experiments, because of the thorough mixing of the water in the bath the total error did not exceed 4-5%. The error encountered using the radiometric method to calculate the rate of photosynthesis was about 10%.

Studying the results obtained by comparing the three methods for determining the rate of photosynthesis, we concluded that obtaining average ratios of 0.993 ± 0.125 (in the air-flow and radiometric methods) and 0.998 ± 0.034 (in the manometric and radiometric methods) indicates the close agreement of the calculated results for the absorption of CO_2 using these different methods, and errors of 12.5% and 3.4% are completely within the limits of experimental error for the methods compared. From the investigation it also follows that the isotope effect to which some authors give considerable space was not observed in our experiments.

Therefore, after radioactive C^{14} has been introduced into plants in the form of CO_2, we can find the number of mg of C or CO_2 absorbed by the plant, by measuring the comparative activity of the samples prepared from them and also to trace the quantitative distribution and redistribution of the absorbed carbon throughout the plant or in any of the organic compounds in which we are interested.

The present investigation was carried out at the Photosynthesis Laboratory of the V. L. Komarov Botanical Institute, Academy of Sciences, USSR, under the direction of O. V. Zalenskii, to whom the authors are grateful for his direction and help.

SUMMARY

By introducing radioactive carbon C^{14} in the form of CO_2 in a nutrient medium and measuring the relative activity of preparations made from the plants one can determine the absolute amount of C or CO_2 in absorbed by the plants and also study the quantitative distribution and redistribution of the absorbed carbon in the plant or in different organic compounds.

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ERRATA

Plant Physiology, Vol. 5, No. 1,
January-February 1958

Page	Line	Reads	Should read
2	29	root	cortex
3	5	For ... out	In the case of the analysis of the radioactive samples of the cortex, we carried out an additional refining of the solution of the ion-exchanged resins distilled in the vacuum.
4, 6, 6, 10	Tables 1, 2, 3, 5	dry weight	wet weight
5	3	Chromatographic ... (4:1:5).	Chromatographic separation on paper made it possible to find in the cortex of both plants, besides the glucose, fructose, and sucrose, a group of oligosaccharides, for which data are given separately in Table 1 on the concentration of the most simple and close-to-sucrose oligosaccharide (first), a more complex but still active oligosaccharides (second), and groups of more polymeric compounds arranged near the start when distilled in a mixture of n-butanol, acetic acid, and water (4:1:5).
15	Table 3	The Effect ... Tissues	The Effect ... Tissues (in % of wet weight)
20	31	sulfate	nitrate
27, 28	33	Distilled ... up.	Distilled water was added daily to the nutrient solution of each container up to the original level and the solution was agitated.
38	Table 1, 26, 27	aportovoi	Oporto
39	10	"	"
40	Table 2	"	"
41	Table 4	Water ... Pear	Water ... Pear (in g/100 g fresh leaves)
42	Table 6	Sugar ... Grafts	Sugar ... Grafts (in % of wet weight)

Page	Line	Reads	Should read
42	Table 6	aportovoi	Oporto
43	Table 7	"	"
56	6	A ... plants.	In this connection, the study of the passage through the vernalization stage for winter varieties in field conditions has great significance, because this would make it possible to control successfully the characteristics of winter resistance of plants.
63	18	true	top
64	5	part of a reserve	role of an intermediary
67	1	properties	processes
	7	The . . . resistant.	The plants were in the stage of deep quiescence for the entire period.
	20	physiological	meteorological
69	[9]	Izblakova	Iablokova
71	4	in many perennial	in perennial
77	7, 8	...; the difference ... content.	the different soils and fertilizers effect only the yield and not the concentration of nitrogen.
	14, 15	The ... University.	The experiments were carried out on the drained peat of the Sarensk experimental hydro-development Station, Rovensk region, and on the mineral soil of the botanical garden of the I. Franko State University, L'vov.
	author's name	A. S. Palamarchik	A. S. Palamarchuk
88	16	wholesome	expedient
89	11	The disturbance	The minimum disturbance
93	37	carbon	carbohydrate
	40	fungicide	phytoncide

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